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ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"

Diagnosis of Rift Valley fever in animals: laboratory aspects and more

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Regional Workshop on Rift Valley Fever surveillance in Northern African countries

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Definition

- Rift Valley fever (RVF) is an **arthropod-borne viral zoonosis** that primarily affects animals but also has the capacity to infect humans. Infection can cause **severe disease** in both animals and humans. The disease also results in significant **economic losses** due to death and abortion among RVF-infected livestock.

(WHO, 2018)

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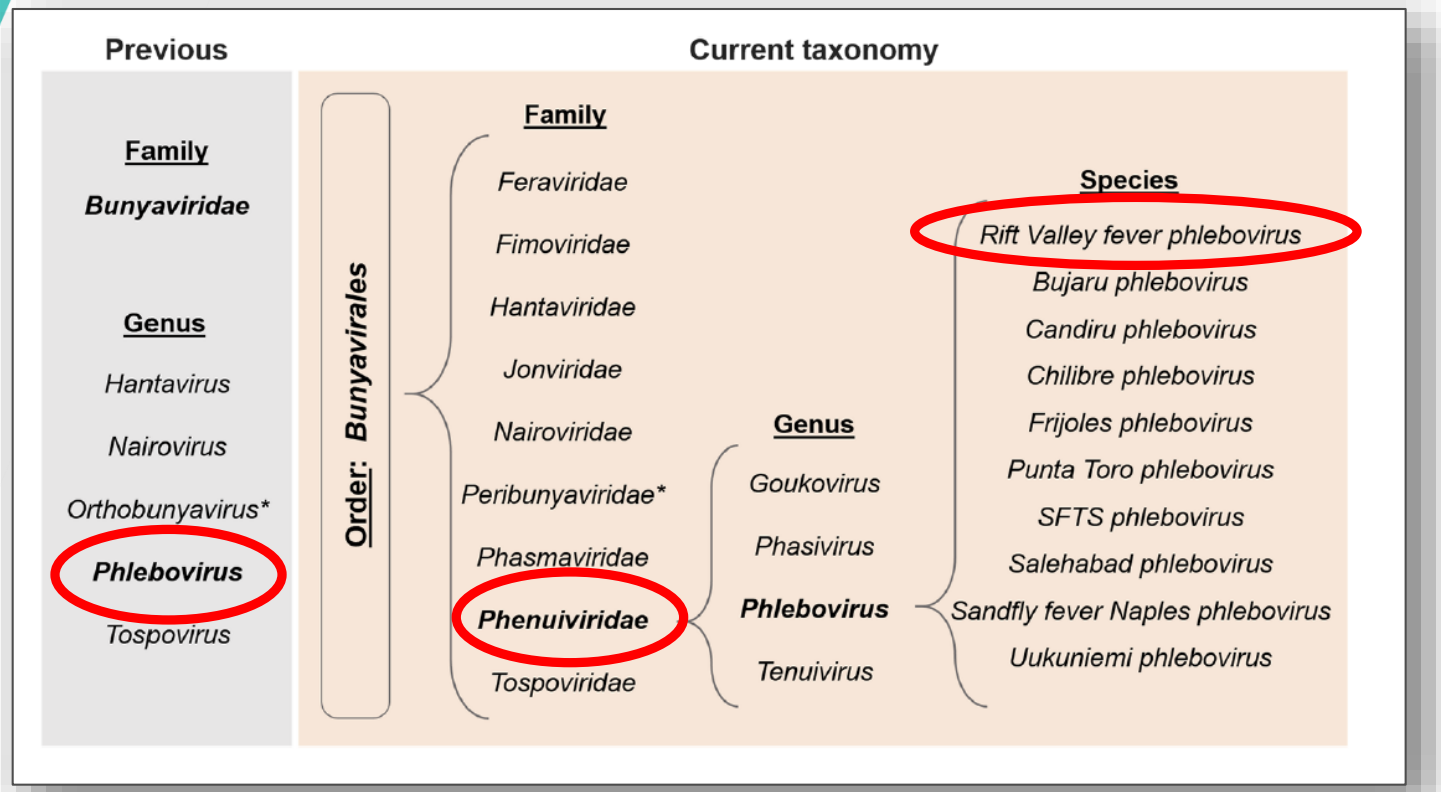
Virus



- In 2016 it was reclassified by ICTV into *Bunyavirales* order

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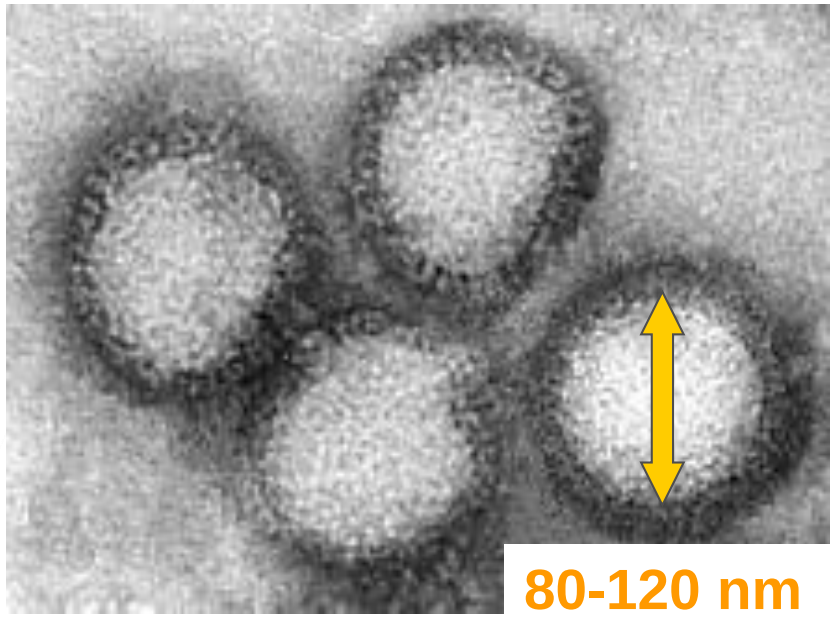
RVF phlebovirus



- Characteristic of the virus:
  - Spherical enveloped virus of 80 - 120 nm
  - Two surface glycoproteins Gn and Gc

(WHO, 2018)

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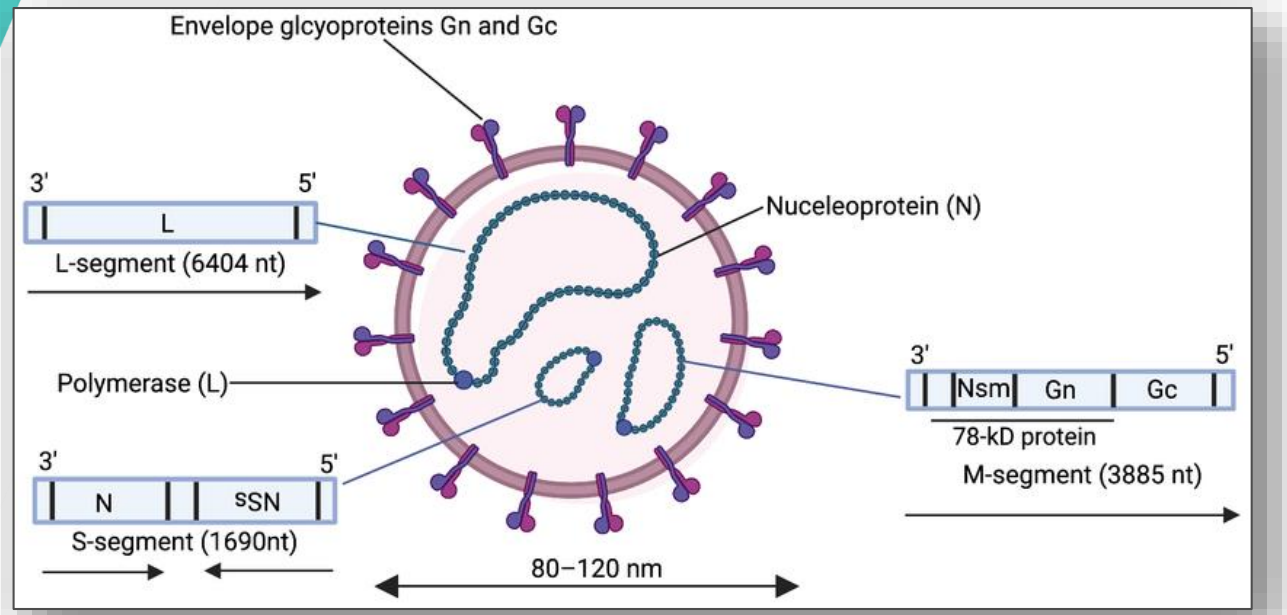
RVF phlebovirus

Kitwandwe *et al., 2022*



Genome composed by 3 segments of ss RNA:

- **L-Large segment**
  - *RdRp*
- **M-Medium segment**
  - *Non-strucutural M (NSm's)*
  - *Glycoproteins (Gn, Gc)*
- **S-Small segment**
  - *Nucleocapsid protein (NP)*
  - *Non-strucutrual S (NSs)*

(WHO, 2018)

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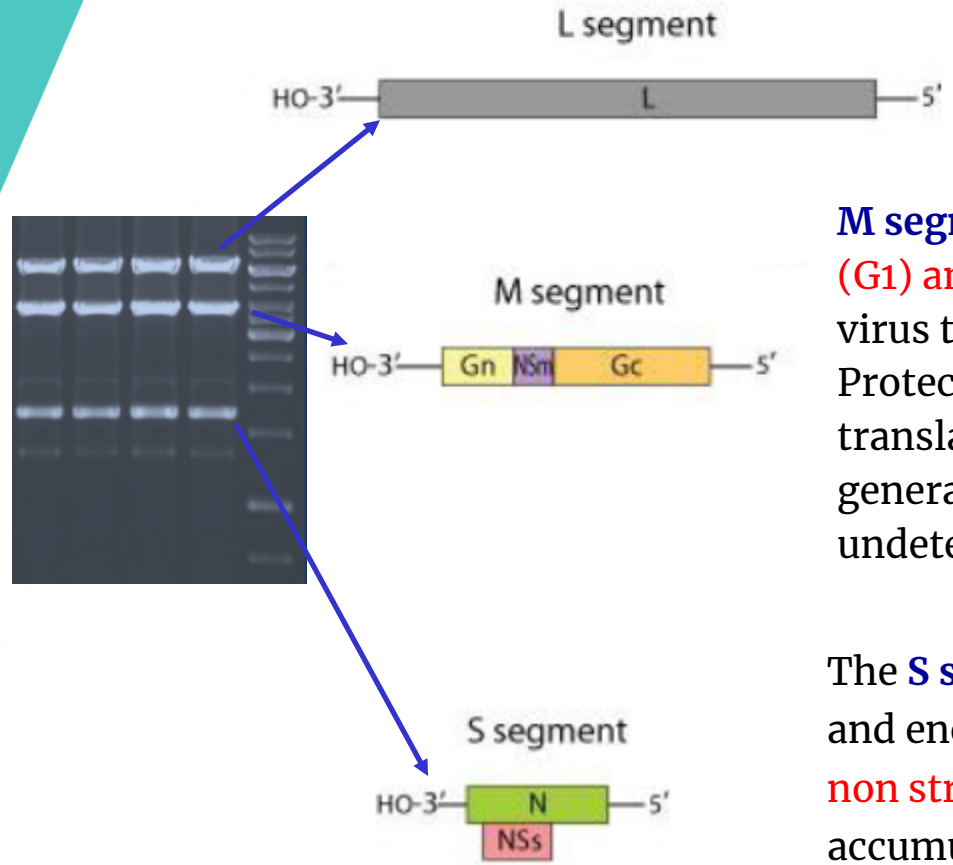
RVFV Structure



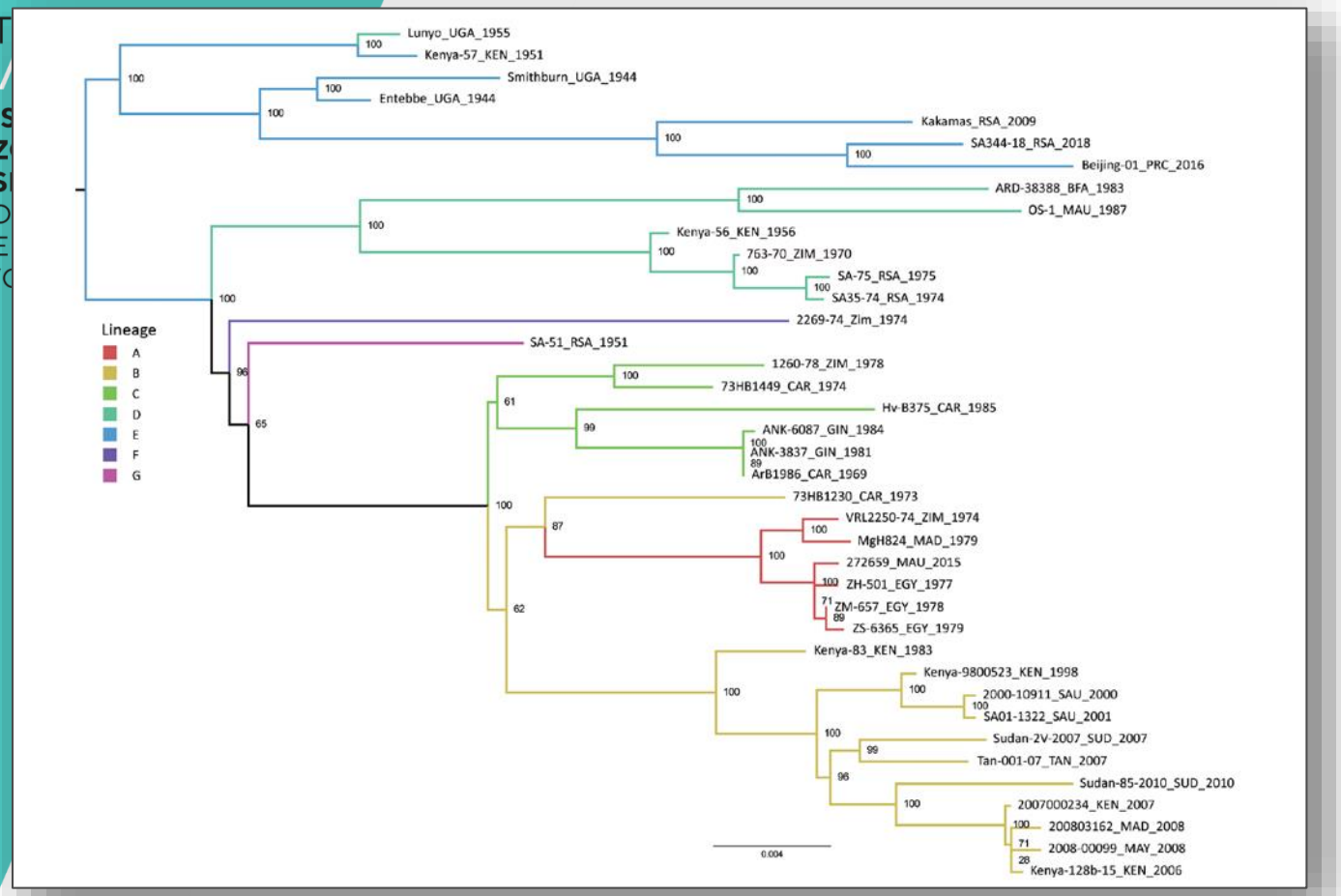
The **L segment** expresses the RNA dependent RNA polymerase L

M segment expresses the precursor to the glycoproteins GN (G1) and GC (G2) which are responsible for the fixation of the virus to the host cells, targets of the immune response. Protective antibodies are against these glycoproteins. Post-translational 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)

- ✓ Bird et al. (2007) J. Virol. 81: 2805-16
- ✓ Bird et al. (2008) J. Virol. 82: 11152-66
- ✓ Carroll et al. (2011) J. Virol. 85: 6162-7
- ✓ Grobellar et al., (2011) EID. 17(12): 2270–2276

RVF phlebovirus



Jansen van Vuren *et al.*, 2019 *EID* 25(2): 338-341

Virus remarkably stable genetically and antigenically

- *maximum diversity among circulating strains limited to 4% (nt level) (S & L) and 5% (M)*
- *7 to 15 distinct lineages/geographic clustering*
- *segment reassortment*
  - *none observed with other phleboviruses*
  - *only 3-4 examples among RVFV lineages from field isolates/specimens*
  - *Little evidence of recombination*

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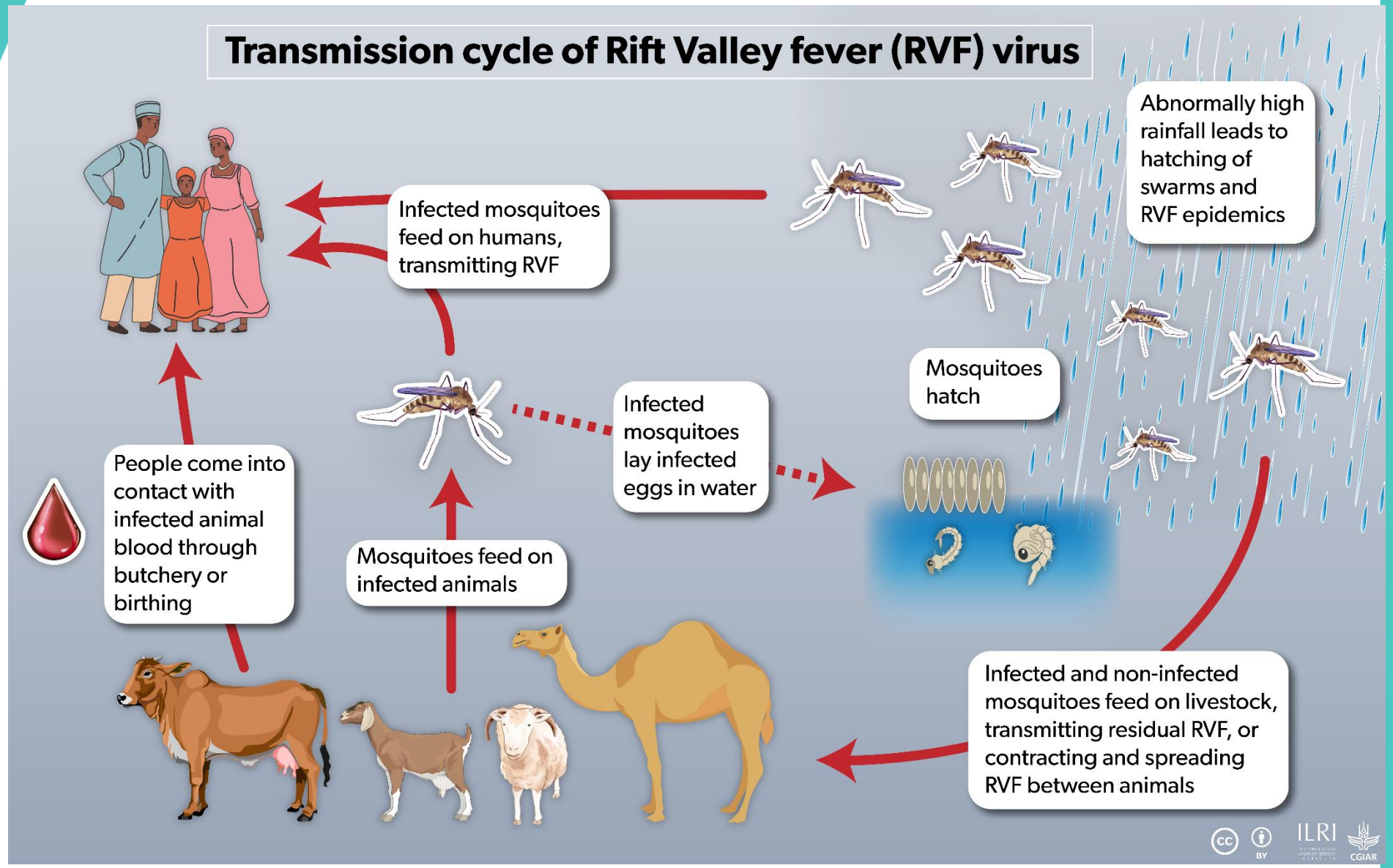

RVF transmission cycle (illustration credit: ILRI/Annabel Slater)

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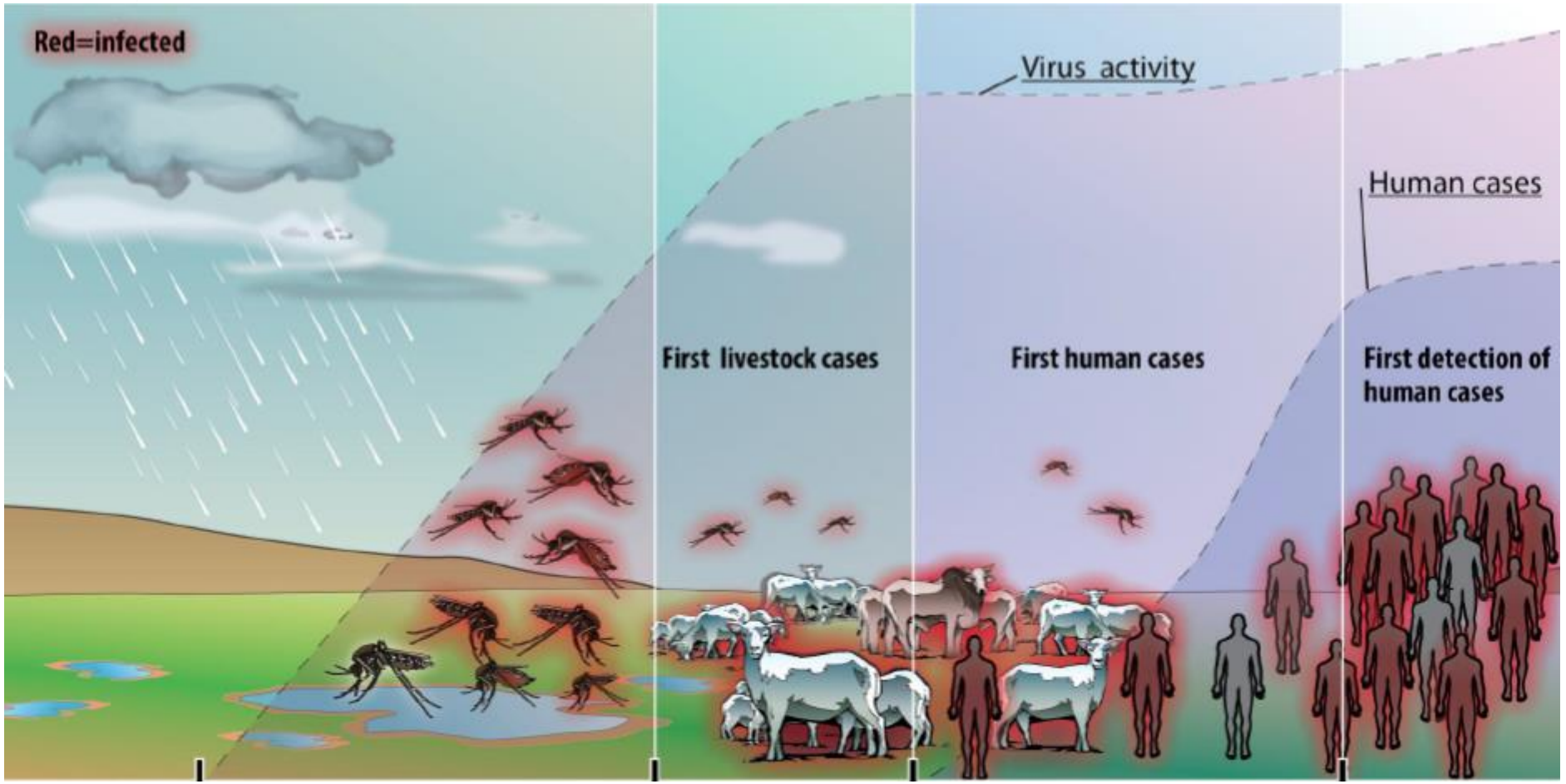
RVF life cycle



Heavy rains and flooding, emerging of infected mosquitoes

Amplification of virus activity for 6-8 weeks before detection of first human case

Start of contain and control programs

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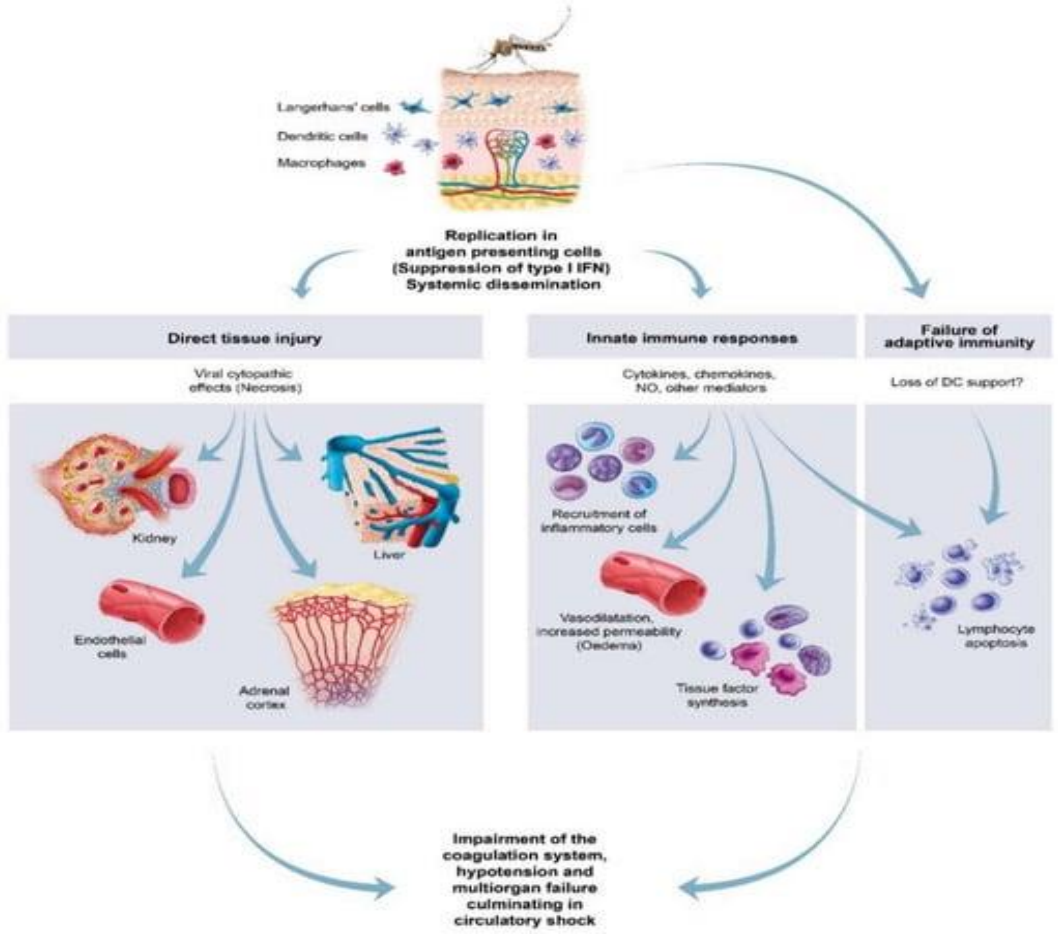
Pathogenic Mechanism



Bray, M. Pathogenesis of viral hemorrhagic fever. Curr. Opin. Immunol. 2005, 17, 399–403

Mosquito bite

Virus endocytosis by antigen-presenting cells.

Suppression of type I IFN production and necrosis of infected macrophages and dendritic cells

Wide dissemination of viruses.

Necrosis in a variety of tissues and cells together with suppression of both the innate and adaptive immune responses.

Apoptosis of lymphocytes through mediator effects and loss of dendritic cell support.

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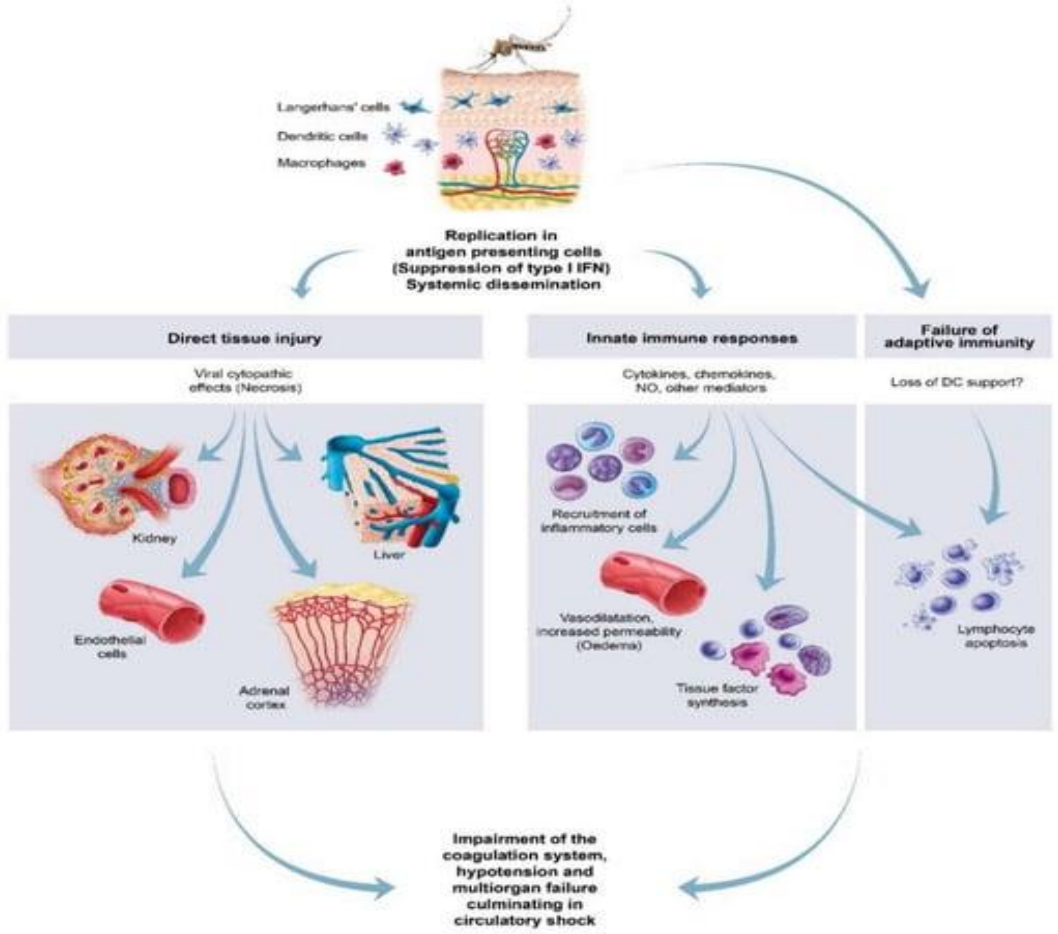
Pathogenic Mechanism



Failure of the immune response exacerbated

Excessive pro-inflammatory cytokine and chemokine response

Increased microcirculatory dysfunction through the action of inflammatory mediators.

Impairment of the coagulation system results in widespread haemorrhages.

Fatal outcomes result from multiorgan failure, oedema in many organs (including the lungs and brain), hypotension and circulatory shock.

Bray, M. Pathogenesis of viral hemorrhagic fever. Curr. Opin. Immunol. 2005, 17, 399–403

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RVF clinical signs in animal

lambs
kids
sheep
calves
goats
cattle
buffalo
humans
camelids

Clinical signs may widely vary, depending on host species, age, stage of production cycle, level of challenge, immunity etc...

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RVF clinical signs in animal

Morbidity/Mortality

SHEEP MOST SUSCEPTIBLE → CATTLE → GOATS

- Clinical disease especially in exotic breeds
- Indigenous animals generally less susceptible
- Pregnant animal abortion

RVF clinical signs in animal

Cattle

- Adults
  - Usually asymptomatic
  - Fever (24–96 hours), weakness, anorexia, drooling, bloody diarrhea, jaundice
  - Death rate 10%
  - Abortion up to 85%
- Calves
  - Fever, depression, sudden death
  - Death rate 10-70%

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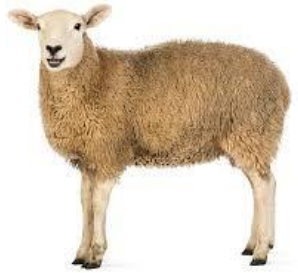
RVF clinical signs in animal

Sheep and goats

Incubation period: < 3 days

- Flock morbidity rate may reach 100%
- High rate of abortion
  - any stage of pregnancy
  - fetus with decomposed appearence

Adults mostly asymptomatic
Fever, jaundice, vomiting and diarrhea
Mortality (5-30%)



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RVF clinical signs in animal

Lambs and goat kids

- Incubation period : 12-36 hrs
- Newborn
  - Peracute form
  - Death - 12 hours to 2 days
  - Less than 1 week old
    - Death rate up to 95%
- Lambs and goat kids over 2 weeks old
  - Death 24-48 hours after the onset of clinical signs
  - Mortality > 20%

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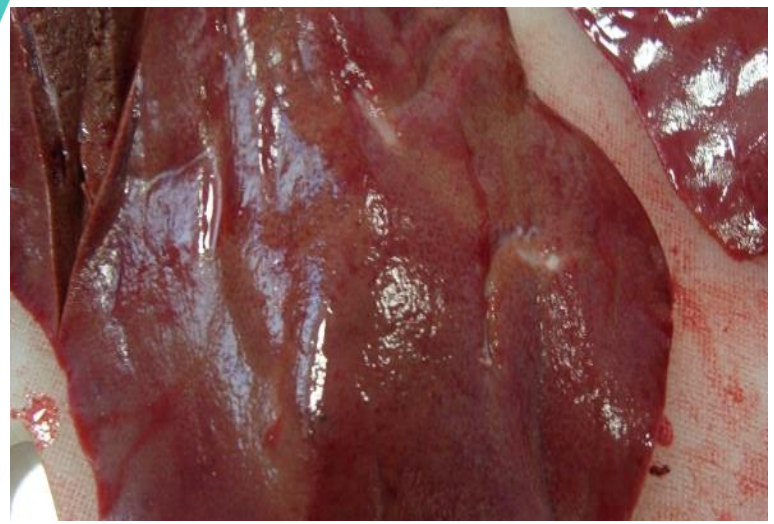
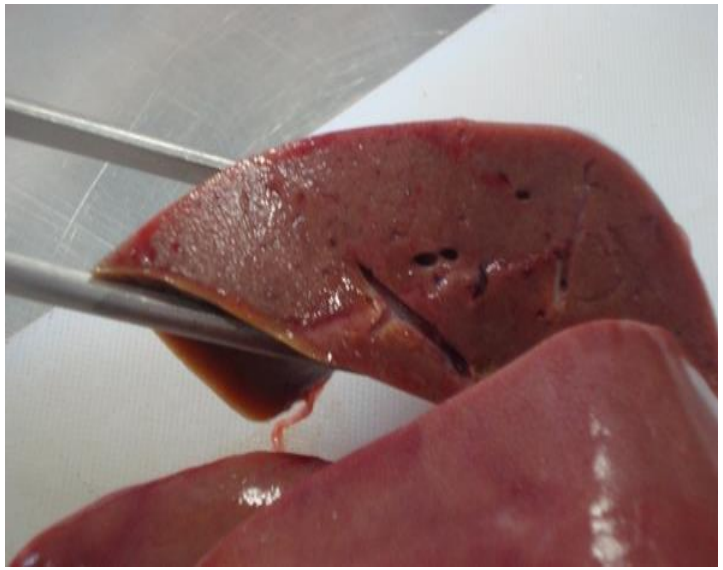
RVF: Gross lesions in adult sheep

Liver



Enlarged, friable;

Discoloured orange-brown; icterus; pin-point reddish to greyish-white necrotic foci



Discrete foci of hemorrhages and necrosis

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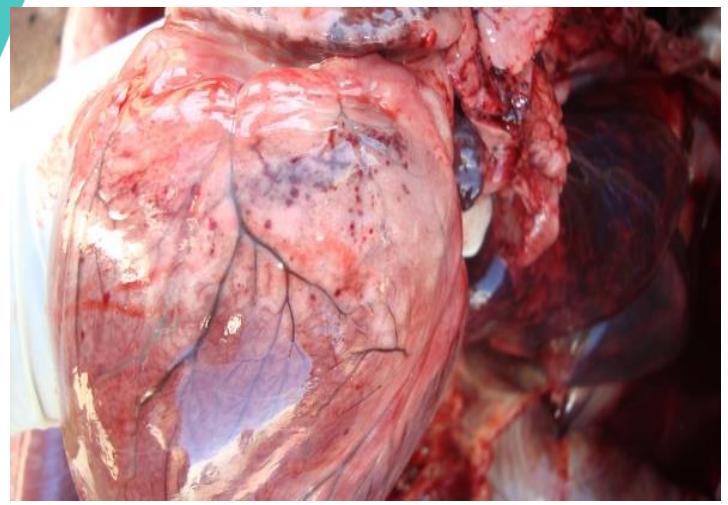
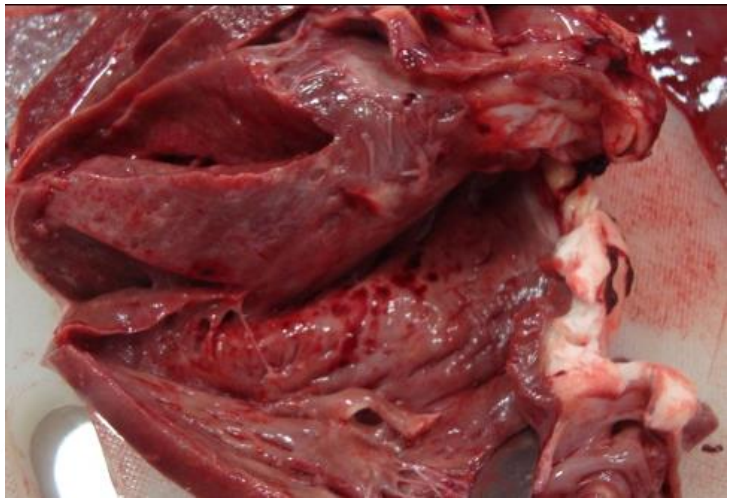
RVF: Gross lesions in adult sheep

Heart



Serosal haemorrhages

Hemorrhages on the epicardium and the endocardium and hydropericardium



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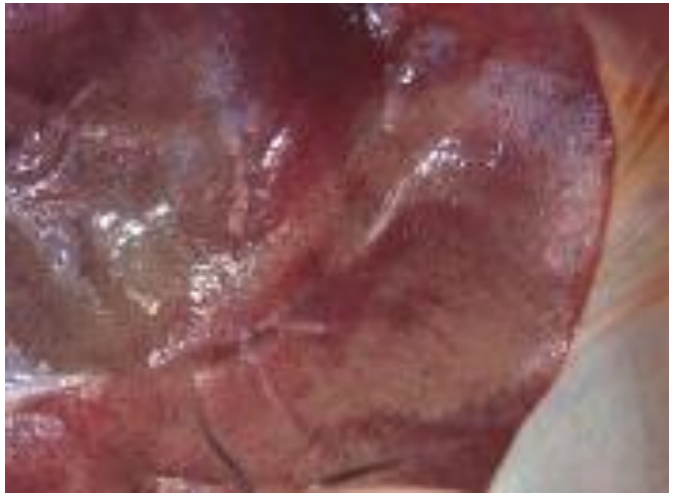
RVF: Gross lesions in adult sheep

Spleen



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

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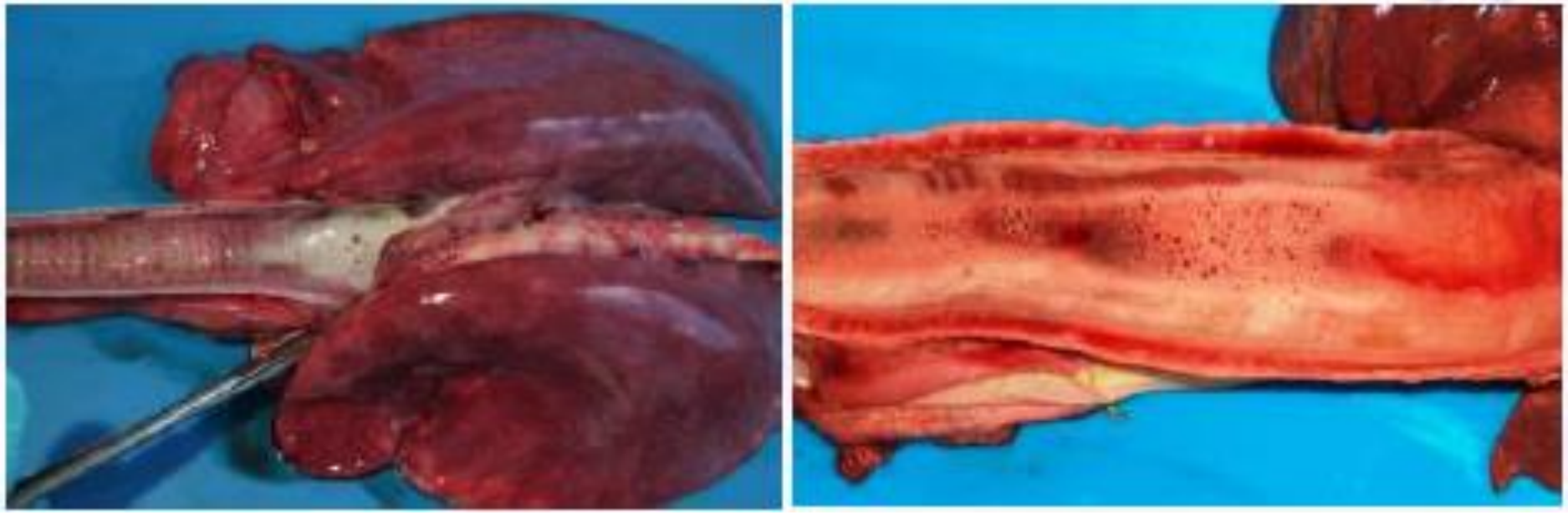
RVF: Gross lesions in adult sheep

Lungs



Oedema and haemorrhages

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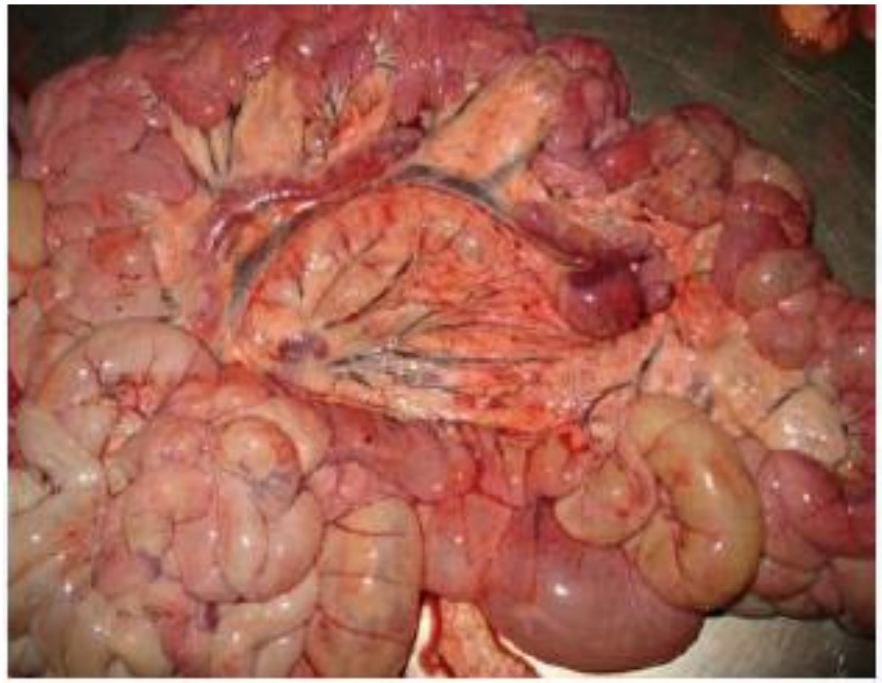
RVF: Gross lesions in adult sheep

Lymphnodes



Marked enlargement and congestion the mesenteric lymph nodes

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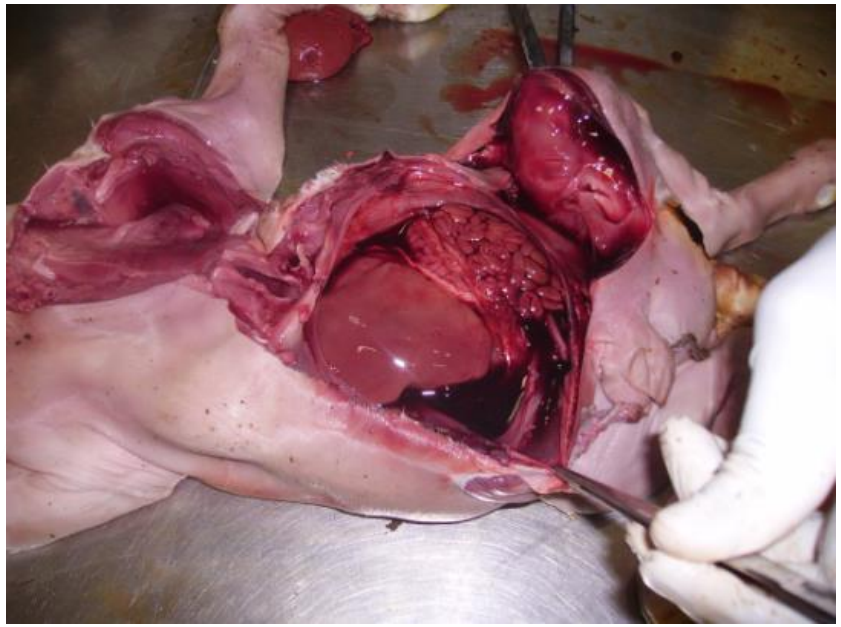
RVF: Gross lesions in newborn lambs



Bloodstained ascitic fluid

Haemorrhages in thymus

RVF clinical signs in animal

Dromedaries

- abortion and perinatal deaths
- hyperacute form, with sudden death in <24 hours
- haemorrhagic septicaemia, severe respiratory distress, nervous symptoms (El Mamy et al., 2011)

Dogs

- Abortion up to 100%
- Severe disease and death in puppies

Cats:

- Death in kittens

Pigs:

- experimentally infected subclinically
- pregnant sows abortion following infection
- viral RNA in oronasorectal swab on 28 DPI : shed RVFV ?

El Mamy AB, Baba MO, Barry Y, Isselmou K, Dia ML, El Kory MO, Diop M, Lo MM, Thiongane Y, Bengoumi M, Puech L, Plee L, Claes F, de La Rocque S, Doumbia B. Unexpected Rift Valley fever outbreak, northern Mauritania. Emerg Infect Dis. 2011 Oct;17(10):1894-6. doi: 10.3201/eid1710.110397. PMID: 22000364; PMCID: PMC3310676.

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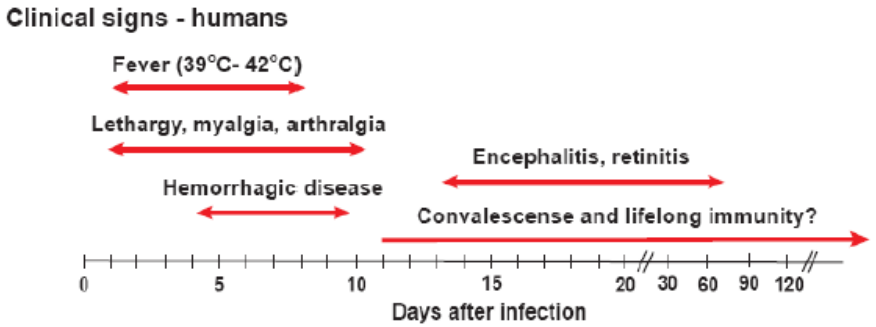
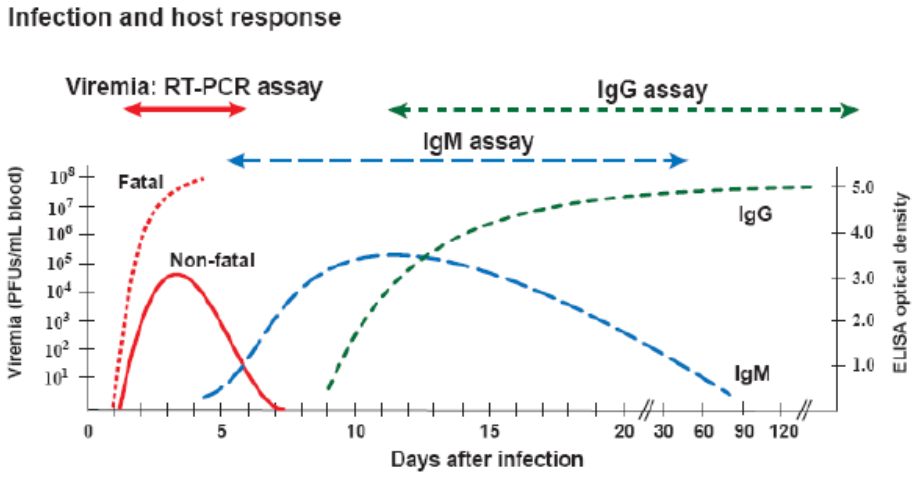
RVF: disease course in humans





Incubation period: 2-6 days on average

Infections

- **Majority of infections are asymptomatic**
- A febrile, flu-like syndrome with myalgia. Recovery in 4-7 days
- Large outbreaks have revealed cases with severe complications:
  - ~ 1% with hemorrhagic fever with high mortality
  - ~ 1-2% with retinal vasculitis
  - < 1% with encephalitis

Kitwandwe *et al.*, 2022

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RVF: disease course in humans

RVF is a major **ZOONOSIS**

Definition: a disease that is naturally transmitted between humans – animals

- >250 zoonosis known
- 75% emerging diseases are zoonosis (OIE, 2004)

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RVF: who is at risk?

- No inter human transmission
- Mosquitoes:
  - Possible but unlikely (Nile delta, 1977)
- Transmission from infected ruminants through:
  - body fluids,
  - aerosol
  - Infected tissues (fetus, placenta. . .)

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RVF: who is at risk?

- Breeders, particularly the members of the family who manipulate the animals (milkers…)

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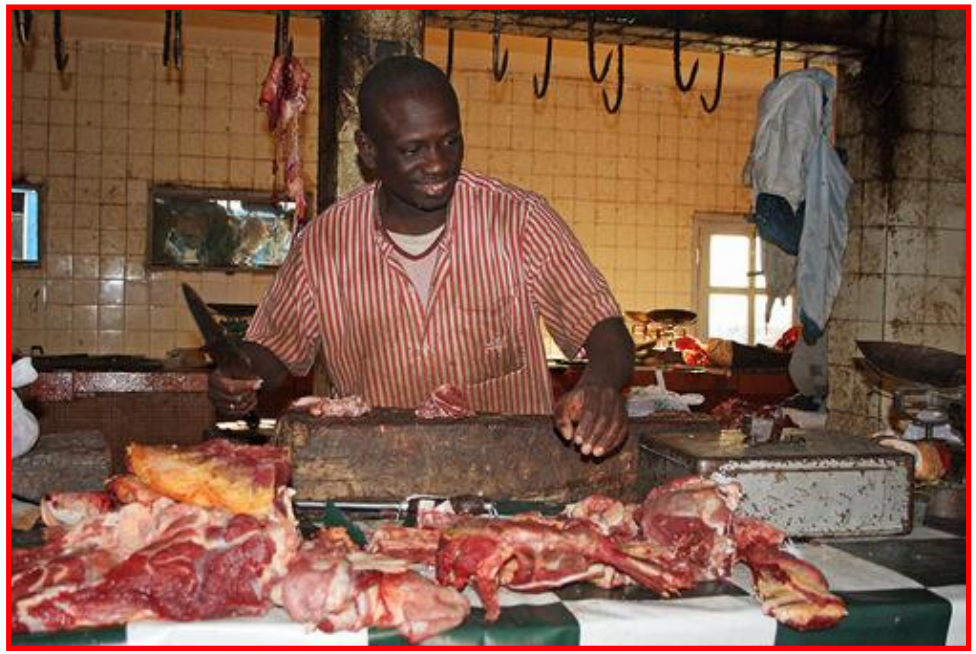
RVF: who is at risk?



- Breeders, particularly the members of the family who manipulate the animals (milkers…)
- Butchers, slaughterers

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- Breeders, particularly the members of the family who manipulate the animals (milkers…)
- Butchers, slaughterers
- Slaughtering of viraemic animals

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RVF: who is at risk?

- Breeders, particularly the members of the family who manipulate the animals (milkers…)
- Butchers, slaughterers
- Slaughtering of viraemic animals
- Veterinarians and field technicians

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RVF: who is at risk?

- Breeders, particularly the members of the family who manipulate the animals (milkers…)
- Butchers, slaughterers
- Slaughtering of viraemic animals
- Veterinarians and field technicians
- Laboratory workers

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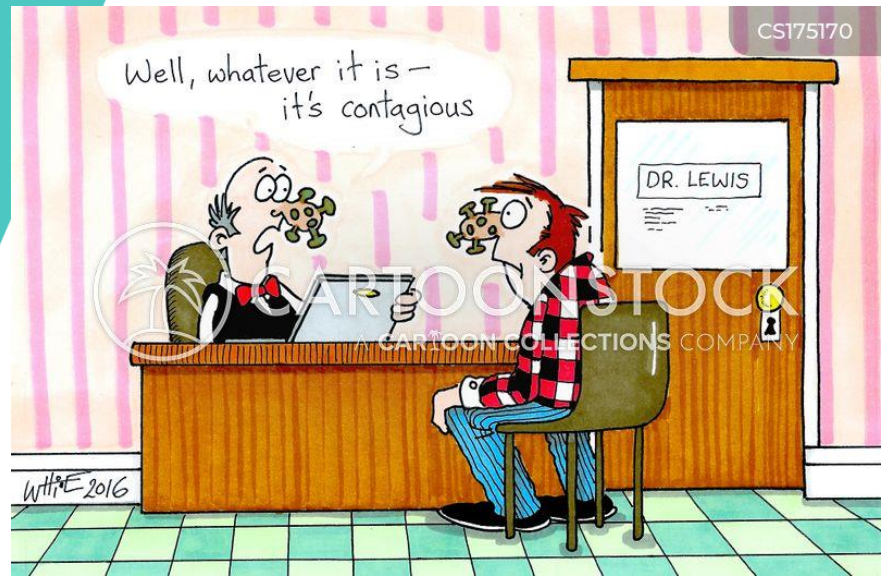
RVF suspect: key features

When RVF should be suspected?



- ✓ Animals
  - Introduction from endemic areas
  - Multiple animals are affected at the same time
    - sudden abortion at any stage of pregnancy in more than one animal;
    - clinical signs such as high fever (adult) and often death in young animals;
    - Recurrent liver lesions at necroscopy.
- ✓ Favourable ecoclimatic conditions
  - Periods of heavy rainfall or flooding associated with increase of mosquitoes population
- ✓ Human
  - Influenza-like sindrome

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RVF: from suspect to diagnosis

Conduct laboratory tests to confirm (or exclude) the presence of the disease.

The most important infection route for humans is through **mucous membrane exposure**, or **inhalation** of viral particles.

Wear protective **personal protective equipment** (PPE) when dealing with animals and/or their secretions, especially in high risk areas and risk situations (e.g. when handling tissues from animals that have aborted).

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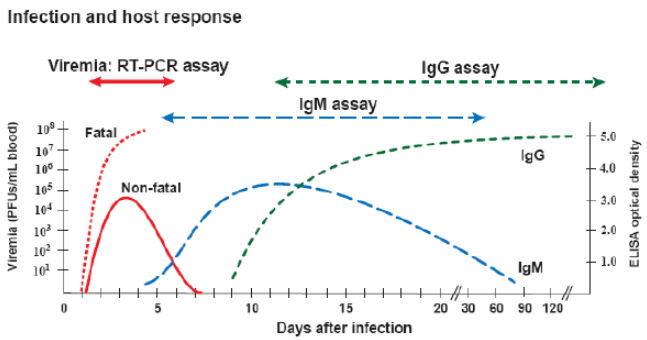
RVF: from suspect to diagnosis



Sample collection

Choose the test according to the time of sampling and the purpose of testing

- Blood
  - Recent infection, animals with fever
- Organs
  - Aborted foetuses
  - Carcasses
- Serum
  - Aborted (and non) animals

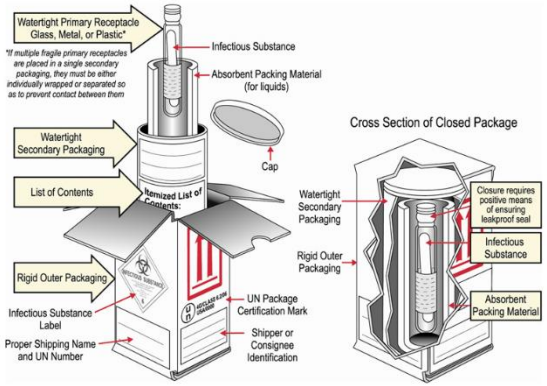
Quality of the specimen and transport/storage conditions will affect the laboratory diagnosis

RVF: from suspect to diagnosis

Sample collection

- Appropriate containers
  - vacutainer tubes (EDTA), leak proof containers

- Biosecure packages



Rapid transfer to the laboratory

The collection of specimens and their transport should comply with the recommendations in *Chapter 1.1.2* Collection, submission and storage of diagnostic specimens and *Chapter 1.1.3* Transport of specimens of animal origin of the WOAH Terrestrial Manual.

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Laboratory diagnosis

RVFV: resistance

- RVFV can be inactivated by **formol**, **beta propriolacton** (1/1000), **sodium hypochlorite** (residual chlore >5ppm)
- Stable with a pH between 6.2 and 8, inactivation with a pH <6.2
- Heat stability: temperature of 56°C for 3 hrs to inactivate
- Stable several months at 4°C in blood and sera, 1 month at -20°C

Biosafety level 3 laboratory or cabinet for:

-isolation of the virus on cell culture,

-neutralisation test

-RNA extraction from field strains

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Laboratory diagnosis

Biosecurity

Concentrations of RVFV in blood and tissues of infected animals are often very high. Diagnostic specimens could be **highly infectious**.

BSL-3 practices, containment equipment and **facilities** are recommended to process infectious material. It is important to **dispose safely of carcasses and aborted material** from suspected cases of RVF

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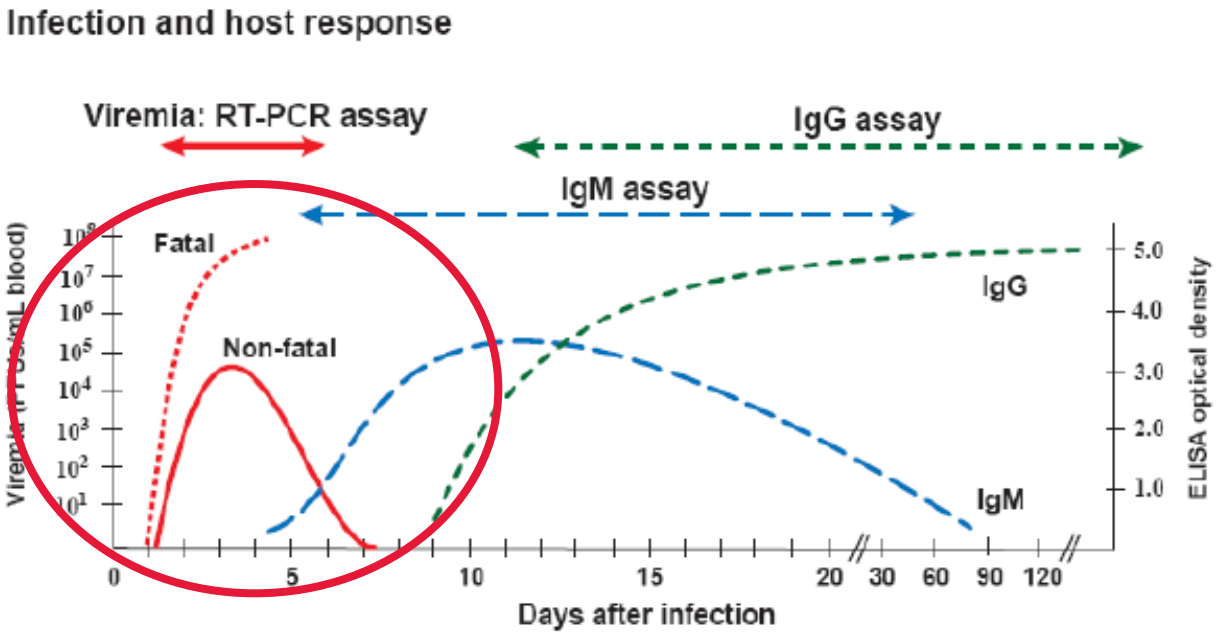
Laboratory diagnosis

Virus detection



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Laboratory diagnosis

Virus detection

Method	Purpose					
	Population freedom from infection (unvaccinated animals)	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases[a]	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection and identification of the agent[b]						
Virus isolation in cell culture	–	–	–	+++	+	–
RT-PCR	–	++	–	+++	+	–
Antigen detection	–	++	+	++	+	–
Histopathology with immuno-histochemistry	–	–	–	++	–	–
Detection of the agent-specific immune response						
ELISA	+++	++	+++	++	+++	+++
VNT/PRNT	+++	+++	+++	++	++	+++

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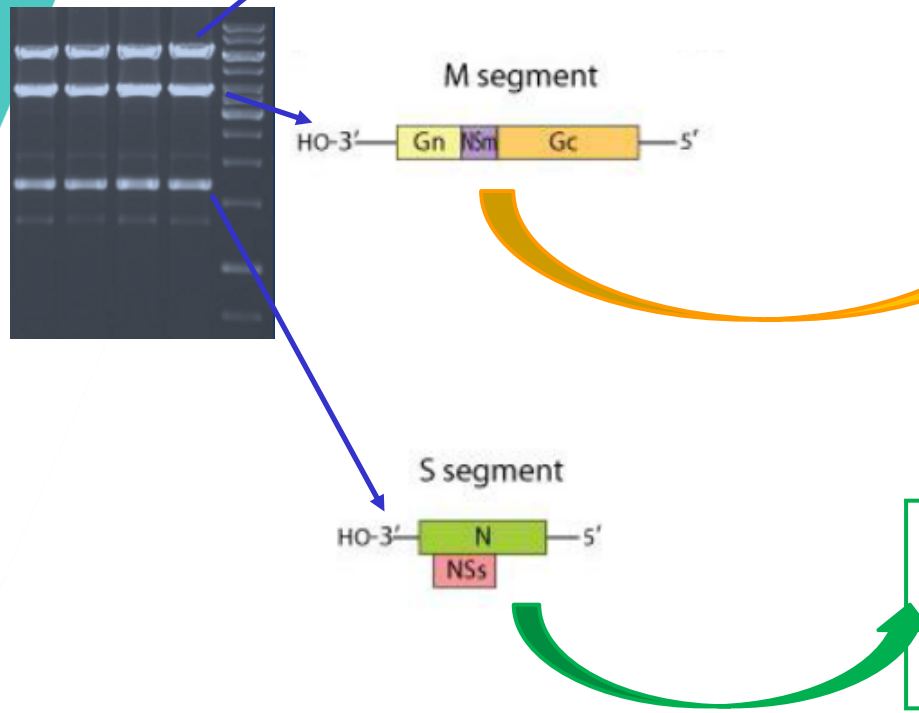
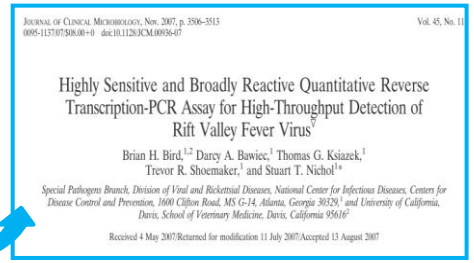
Laboratory diagnosis

Virus detection





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0095-1137/07/$08.00+0 doi:10.1128/JCM.00936-07

Highly Sensitive and Broadly Reactive Quantitative Reverse Transcription-PCR Assay for High-Throughput Detection of Rift Valley Fever Virus▽

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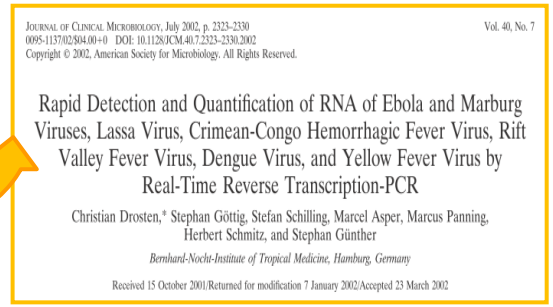

Journal of Clinical Microbiology, July 2002, p. 2323–2330 Vol. 40, No. 7
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.7.2323–2330.2002
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Rapid Detection and Quantification of RNA of Ebola and Marburg Viruses, Lassa Virus, Crimean-Congo Hemorrhagic Fever Virus, Rift Valley Fever Virus, Dengue Virus, and Yellow Fever Virus by Real-Time Reverse Transcription-PCR

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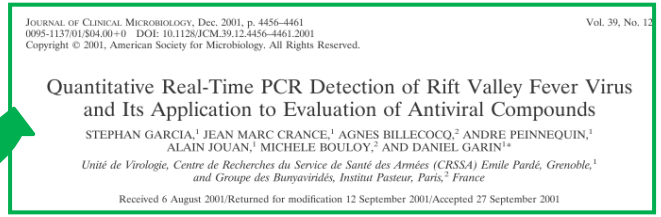
Received 15 October 2001/Returned for modification 7 January 2002/Accepted 23 March 2002



Journal of Clinical Microbiology, Dec. 2001, p. 4456–4461 Vol. 39, No. 12
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.12.4456–4461.2001
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Quantitative Real-Time PCR Detection of Rift Valley Fever Virus and Its Application to Evaluation of Antiviral Compounds

STEPHAN GARCIA,[1] JEAN MARC CRANCE,[1] AGNES BILLECOCQ,[2] ANDRE PEINNEQUIN,[1] ALAIN JOUAN,[1] MICHELE BOULOY,[2] AND DANIEL GARIN[1]*

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RT-PCR: different genome segments…

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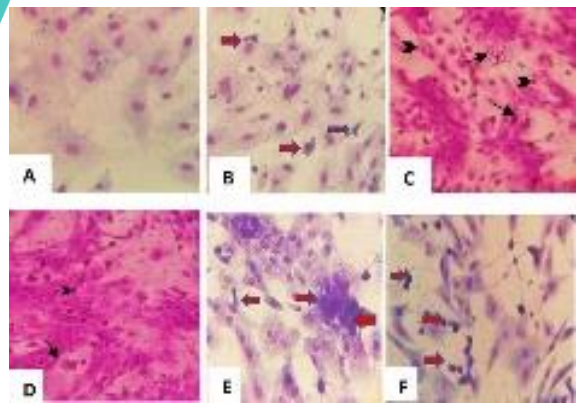
Laboratory diagnosis

Virus detection



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Isolation in cell culture

RVFV can be isolated in a number of common cell cultures: **Vero**, **BHK-21** and mosquito cells. Virus isolation is very **sensitive** and **specific** to confirm the presence of RVFV.

Once infected monolayers cytopathic changes are visible after 2- 5 days post inoculation.

Confirmation should be performed by immunostaining or RT-PCR.

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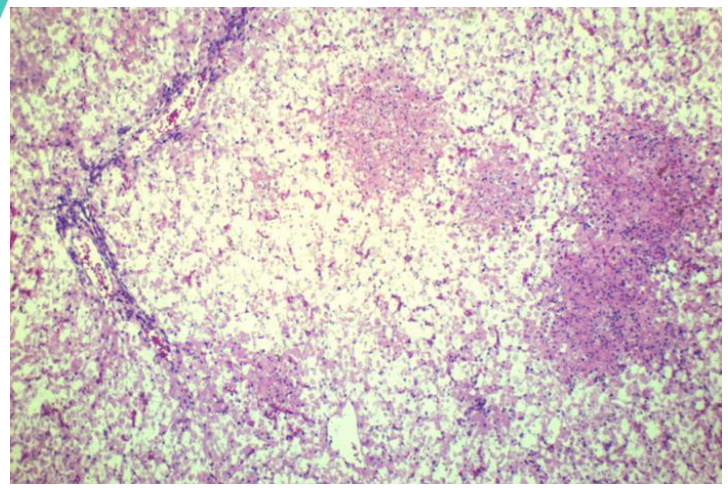
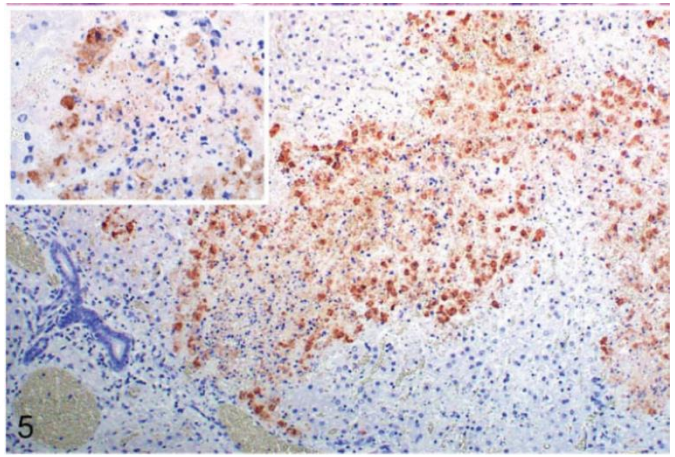
Laboratory diagnosis

Virus detection





Antigen capture ELISA (new LFD)

Allows viral detection from spleen and liver tissues of domestic ruminants

Histopathology : Immunohistochemistry

Histopathological examination of the liver reveals characteristic cytopathology.

Immunostaining allows the specific identification of RVF viral antigen in tissue.

Specimens placed in neutral buffered formaldehyde in the field is inactivated and **does not require a cold chain.**

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Laboratory diagnosis

Virus detection

Focus on Rift Valley Fever

RVF PEN-SIDE TEST: Reliable confirmation of RVF diagnosis in the field

ID Rapid® Rift Valley Fever Antigen

The **ID Rapid® Rift Valley Fever Antigen** is a rapid pen-side test for the detection of RVF virus infection. The technology is based upon reagents from CIRAD, Montpellier, France.

- Results in under 15 minutes directly in the field
- Results are reliable
- Test can be used on whole blood, plasma or serum
- Validated on cattle, small ruminant and camelid samples
- All reagents are provided and ready-to-use

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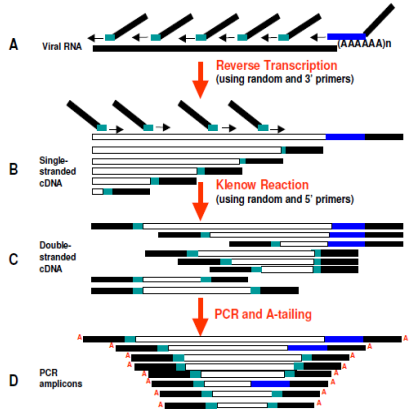
Laboratory diagnosis

Virus detection

Etosha National Park

2 serum samples positive
by Real time RT-PCR (C_t 15, 17)

→ RNA →



Sequencing Indipendent Single primer Amplification

Whole Genome Sequencing by NGS

←

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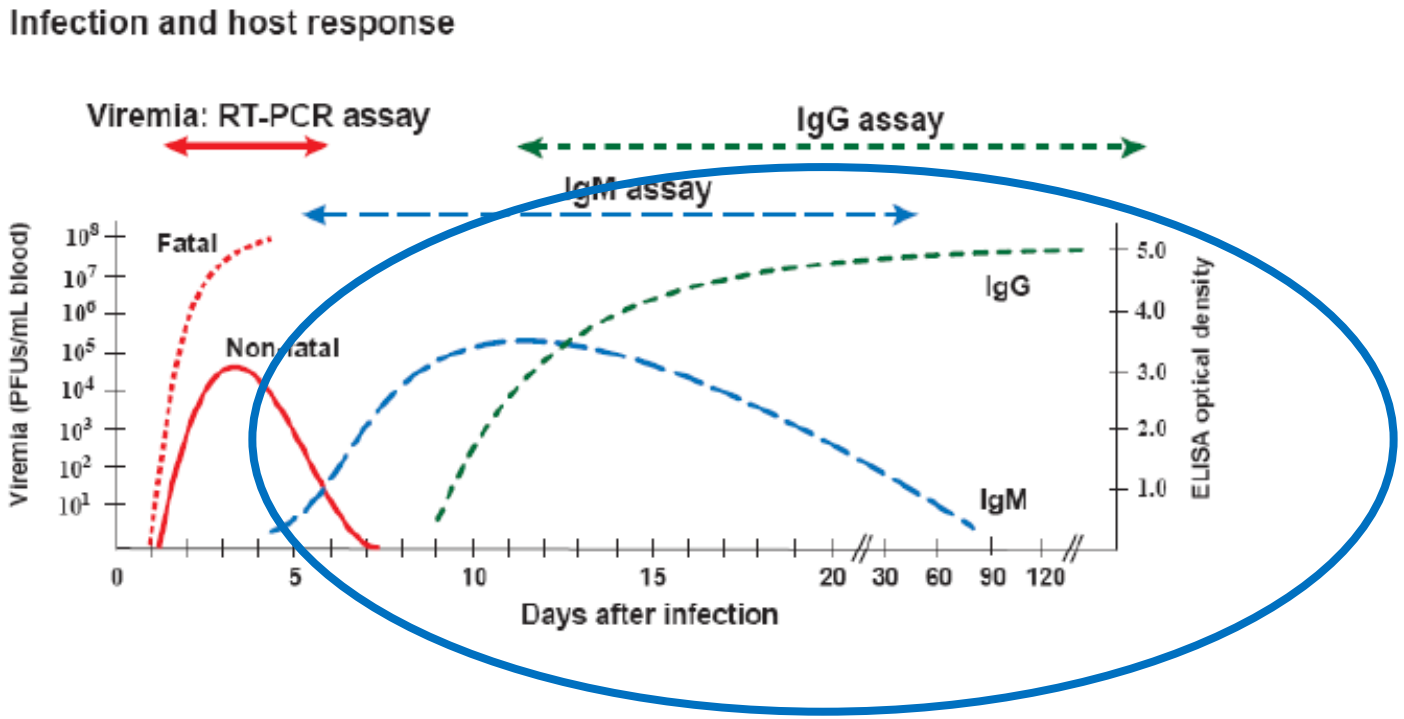
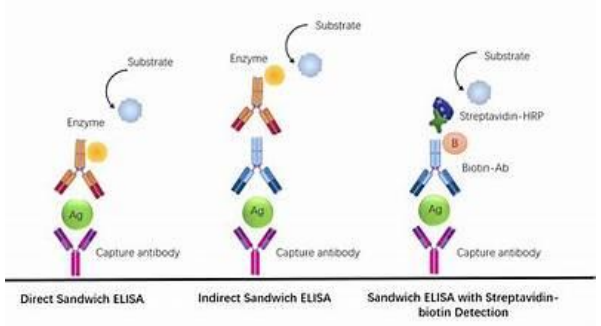
Laboratory diagnosis

Antibody detection



Laboratory diagnosis

Antibody detection



Enzyme Linked Immunosorbent Assay

- ✓ Reliable and sensitive test to detect antibodies against RVFV.
- ✓ IgM antibodies (from 8 – 90 dpi)
- ✓ IgG antibodies (from 10->180 dpi)
- ✓ The appearance of antibodies generally coincides with the gradual disappearance of the virus in the blood.

Serum or blood samples may contain live virus (10^{10} RNA copies/ml sheep - 10^{8} RNA copies/ml cattle) and must be inactivated prior to testing.

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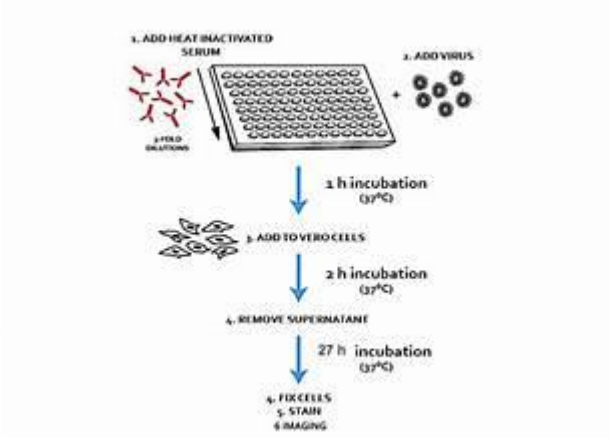

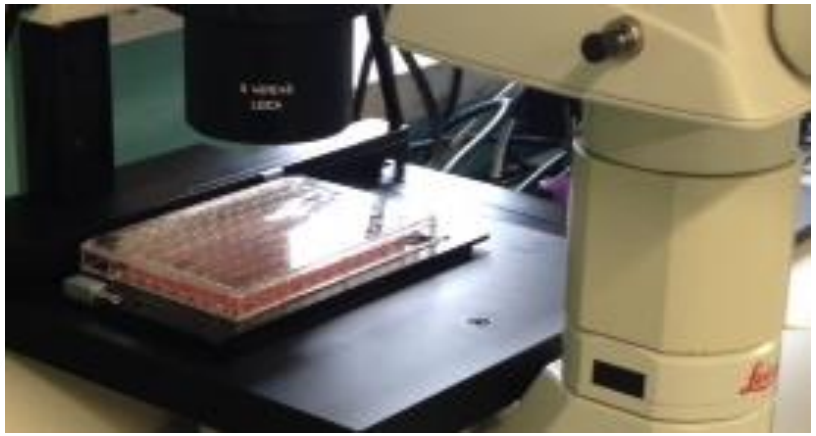

Neutralization test (PRNT/VNT)

- ✓ PRNT/VNT can be used to detect antibodies against RVFV in the serum of any species.
- ✓ NTs are the most specific diagnostic serological test, but using live virus require appropriate biosecurity facilities

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RVF vaccines

	Smithburn live attenuated virus vaccines	Clone-13 live attenuated virus vaccine	MP-12 attenuated virus vaccine	Inactivated virus vaccines
Origin of the isolate	Mosquito isolate, Uganda, 1948	Human isolate, 1974	Egyptian human strain ZH548, 1977	Field strains (South Africa and Egypt) used
Attenuation	More than 200 passages in murine brain	Natural deletion in NSs gene	Mutagen directed attenuation (23 mutations)	Not applicable
Production substrate	BHK cell line	Vero cell line	Vero E6 cell line	BHK cell line
Target	livestock	livestock	livestock	livestock
DIVA policy	No	No	No	No

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RVF vaccines

- Formalinised Rift Valley fever virus with aluminium hydroxide gel as adjuvant for the prophylactic immunisation of cattle, sheep and goats.
- Susceptible animals can be immunised at any age irrespective of the stage of pregnancy and lactation.
- Calves and lambs from immune animals can only be effectively immunised after the age of six months.
- Annual vaccination is recommended

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RVF vaccines

- Freeze-dried, live attenuated Rift Valley fever virus (**Smithburn strain**) for the immunisation of cattle, sheep and goats against Rift Valley fever.
- Animals can be vaccinated at any age (>6 months)
- Full immunity is obtained three weeks after inoculation. A single inoculation usually produces a life long immunity but all animals will not necessarily be completely protected.

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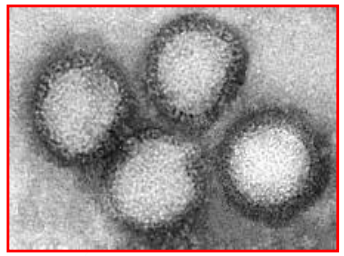
Serological response



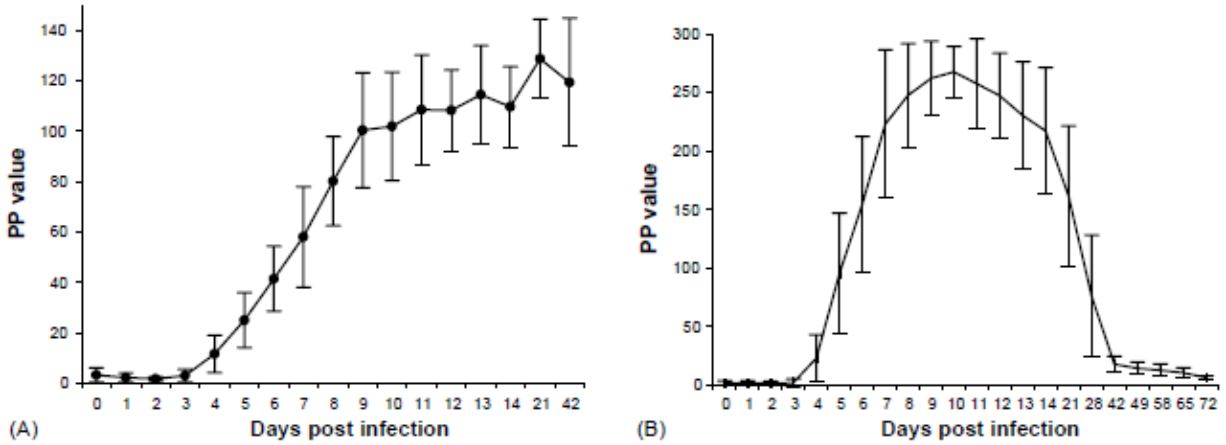
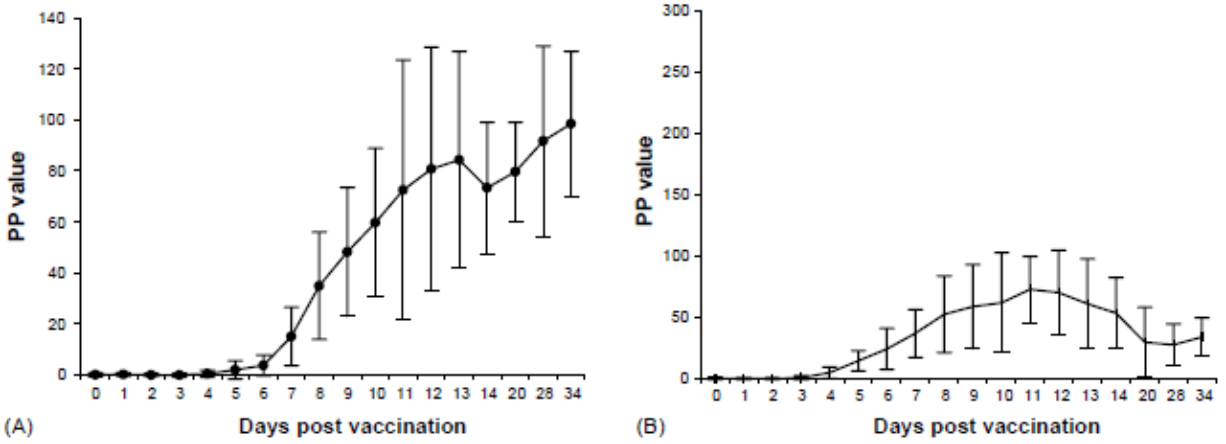


Fig. 2. Mean ± 1 S.D. IgG (A) and IgM (B) responses in sheep ($n = 8$) infected with wild type AR 20368 strain of Rift Valley fever virus.



3. Mean ± 1 S.D. IgG (A) and IgM (B) responses in sheep ($n = 10$) vaccinated with live-attenuated Smithburn strain of Rift Valley fever virus.

Paweska et al., 2003

RVF vaccines

- Freeze-dried, live attenuated Rift Valley fever virus (**Clone 13 strain**) for the immunisation of cattle, sheep and goats against Rift Valley fever.
- Young animals immunised >2 months; >6 months if from vaccinated mother
- Annual vaccination
- Parent strain (74HB59) isolated in Central African Republic from nonfatal human case (Muller et al., 1995)
- Highly attenuated natural RVF mutant (avirulent)
  - 70% deletion (549 nucleotides) within NSs seg
- NSs associated with virulence:
  - Deletions results in high interferon production (Bouloy et al., 2001)

Vaccine 29 (2011) 5771–5777

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Safety and efficacy of Rift Valley fever Smithburn and Clone 13 vaccines in calves

Beate von Teichman[a,*], Anita Engelbrecht[a,1], Gcwalisile Zulu[a,2], Baptiste Dungu[b,3], Anne Pardini[c,4], Michele Bouloy[d,5]

Serum neutralizing antibody titres of calves vaccinated with RVF Smithburn (Group 1) and RVF Clone 13 (Group 2) and challenged with virulent RVF virus. Group 3 served as unvaccinated infected control animals.

Group	Animal no.	Vaccine group	Vaccination				Challenge				
			Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56
1	#1365	RVF Smithburn	0	0	≥512	≥512	≥512	≥512	≥512	≥512	≥512
	#1401		0	0	64	192	≥512	≥512	≥512	≥512	≥512
	#1407		0	0	256	256	256	≥512	≥512	≥512	≥512
	#1412		0	0	128	192	192	≥512	≥512	≥512	≥512
	#1415		0	0	≥512	≥512	≥512	≥512	≥512	≥512	≥512
2	#1366	RVF Clone 13	0	0	24	12	32	≥512	≥512	≥512	≥512
	#1368		0	16	256	32	32	≥512	≥512	≥512	≥512
	#1403		0	8	128	64	32	≥512	≥512	≥512	≥512
	#1408		0	16	64	16	32	≥512	≥512	≥512	≥512
	#1413		0	4	32	64	64	≥512	≥512	≥512	≥512
3	#1367	Control					0	16			
	#1402						0	euth			
	#1404						0	32			
	#1405						0	euth			
	#1406						0	8			

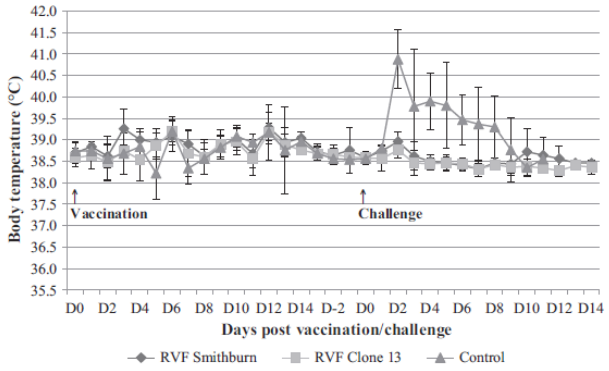


Fig. 1. Mean (±SD) body temperature recordings (°C) for vaccinated and challenged calves.

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Thank you for your attention!

"What did you take away from the meeting?"