Rift Valley fever virus transmission and infection mechanisms

Magnus Evander

Division of Virology, Department of Clinical Microbiology, Umeå University, Umeå, Sweden

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Division of Virology, Umeå University

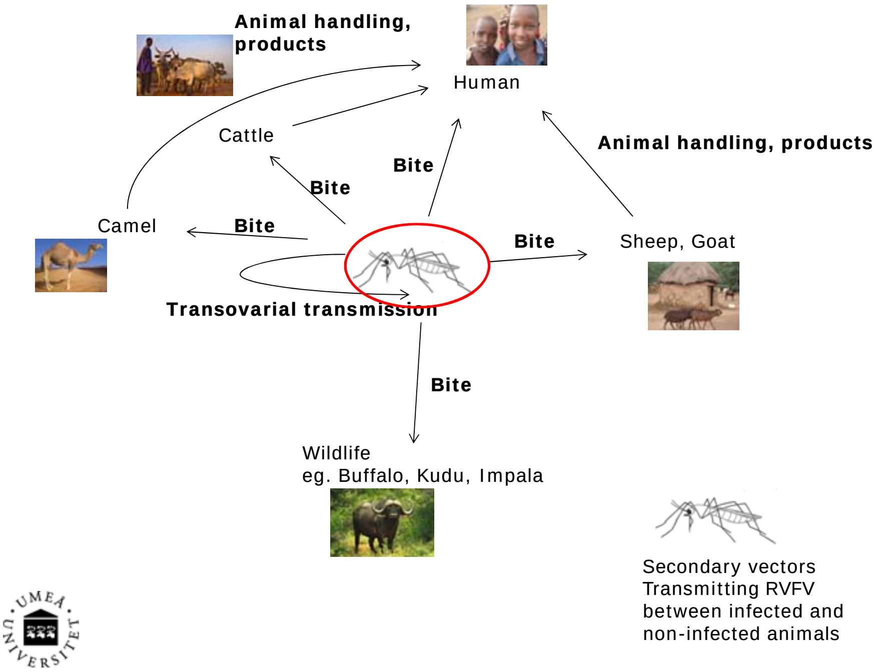
(45 employed researchers, PhDs, technicians, etc)

Group leaders Virology

- Allard Annika – Viruses, water and climate change
- Arnberg Niklas – Adenovirus, Enterovirus, Influenzavirus
- Elgh Fredrik – Prostate cancer
- Evander Magnus – RVFV, mosquitoborne viruses, Hantavirus
- Mei Ya-fang – Adenovirus gene therapy vectors
- Wadell Göran – Antivirals
- Överby Anna – TBE, Flavivirus, (RVFV)

RVF research in Umeå

- Diagnostics, qRT-PCR, sampling
- Infection kinetics in a mouse model
- Vaccines (DNA and virus like particles)
- Early infectious pathway
- Prediction of outbreaks



Human

Animal handling, products

Cattle

Bite

Camel

Bite

Bite

Animal handling, products

Sheep, Goat

Bite

Transovarial transmission

Bite

Wildlife
eg. Buffalo, Kudu, Impala

Secondary vectors
Transmitting RVFV
between infected and
non-infected animals

RVFV transmission cycle - mosquitoes

- Mosquitoes and animals maintain the virus in inter-epidemic periods

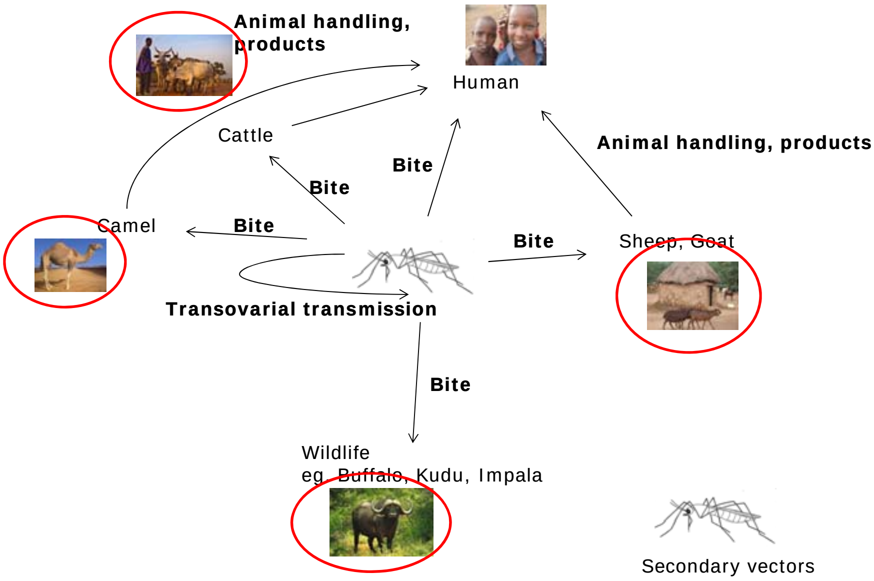
Outbreak
- Unusually heavy and persistent rainfall - flooding
- Transovarially infected adult floodwater *Aedes* species emerge in immense numbers
- RVFV is transmitted to nearby domestic animals (and humans)
- High viremia in the animals
- Colonization of the flooded sites by *Culex, Anopheles, Mansonia* and other species - secondary vector
- Spread to additional animals and humans

Mosquitoes with RVFV

- RVFV isolated from more than 40 species of 8 genera
- *Aedes* mosquitoes are believed to be the primary vector (could depend on geographical location)
- Lab studies – numerous species are susceptible to oral infection – some can then transmit RVFV by bite
- Vertical transmission by *Aedes*, subgenera *Neomelaniconion* and *Aedimorphus*

Marigat district, Kenya, (March 2010)

(photo: Magnus Evander)



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Secondary vectors

RVFV reservoir? Wildlife?

(Evans et al., Epidemiol Infect 2008)

Kenya 1999-2006 – interepidemic period

>15% seropositive

- African buffalo (highest titers)
- Impala
- Waterbuck
- Lesser kudo
- Black rhino

<15% seropositive

- African elephant
- Kongoni

Negative – Lions, Giraffes, Zebras, Warthogs

Kenya 2006-07 – Outbreak

84% seropositivity in

- Gerenuk
- Waterbuck
- Eland

RVFV reservoir? Wildlife?

South Africa 2000-2006, interepidemic period

(La Beaud, Am J Trop Med Hyg, 2011)

- African buffalo 21% seropositivity
- 7% (9/126) seroconverted during the study
- No effect on survival of the buffalo

Animal Disease

Death Rate 100%	Severe Illness Abortion Death	Severe Illness Virus in Blood Abortion	Infection Virus in Blood	Do Not Become Infected
Lambs	Sheep	Monkeys	Horses	Rodents
Calves	Cattle	Camels	Cats	Rabbits
Kids	Goats	Rats	Dogs	Birds
Puppies	Humans	Squirrels	Monkeys	
Kittens				
Some rodents				

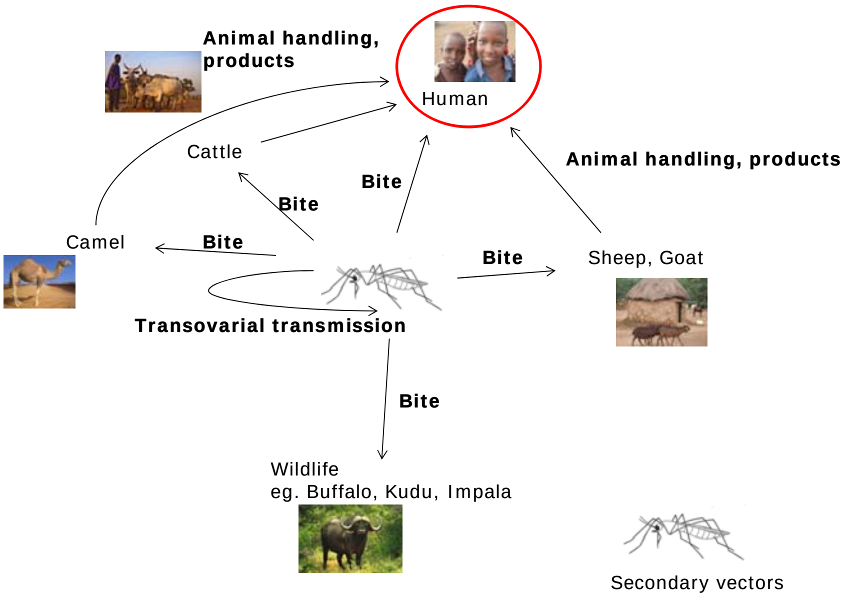
Marigat district, Kenya, (March 2010)

(photo: Magnus Evander)

Transmission to domestic animals and wildlife

- Mosquitoes

- Congenital?
- Milk?
- Other contacts?



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Secondary vectors

Transmission to Humans

- Vector
  - Bite of mosquito infected with RVFV
- Direct Contact
  - Tissues or body fluids of infected animals
  - Handling birthing tissues, meat
- Aerosol
  - Breathing in the virus during slaughter of infected animals or during the birthing process

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Transmission to humans – severe disase

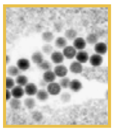
(Anyangu et al., 2010, Am J Trop Med Hyg)

Consumed or handled products from sick animals
Multivariable model **OR 2.53 p<0.0001**

Herdsperson
Multivariable model **OR, 1,77 p<0.0042**

Slaughtered animals, skinned animals, slept outside with herd, milked animals, contact with animal blood, cared for animals during bathing, consumed raw milk - **significant only in bivariable comparison**

RVFV tropism



- RVFV can infect a wide spectrum of tissues in humans, mosquitoes, animals
- Many different cell lines are also infected
- Recently DC-SIGN was suggested as a receptor in immature dendritic cells

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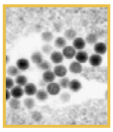
Important questions to address in understanding RVF pathogenesis

1. Route of viral entry to the affected tissues
2. Mechanism that triggers disease

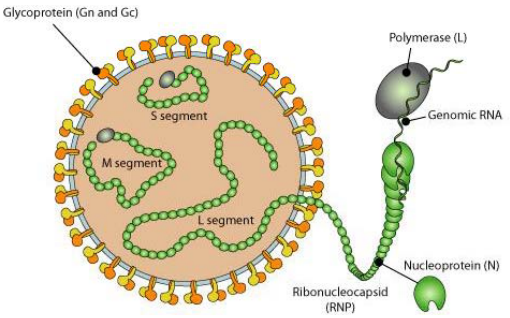
Study of viral entry

Maria Baudin, PhD student



- Identify conditions important for RVFV attachment
- Characterize infectivity pattern of RVFV

Rift Valley fever virus (RVFV)
Bunyaviridae, -ssRNA virus



Segment	**Proteins**	**Function**
L	RNA-dependent RNA polymerase (L)	Endonuclease transcriptase, replicase, RNA helicase
M	Viral glycoproteins (Gn and Gc)	Initial binding to host cell
	Non-structural protein (NSm)	Inhibit apoptosis
S	Nucleocapsid protein (N)	Form ribonucleocapsids that protect the RNA
	Non-structural protein (NSs)	Block interferon response in the host

RVFV is a BSL-3 agent

- RVFV (BSL-3)
- RVFV NSs deleted with a reporter gene (BSL-2) – Can replicate in cell culture
- RVFV virus like particles with a reporter gene (BSL-2) - Can not replicate, only primary transcription

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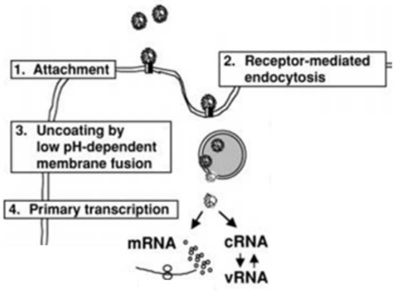
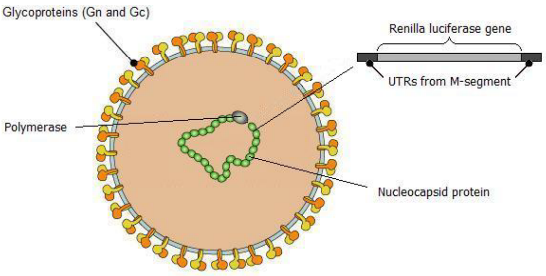
RVF-VLP infection – focus the study to attachment, entry and primary transcription



Renilla luciferase activity

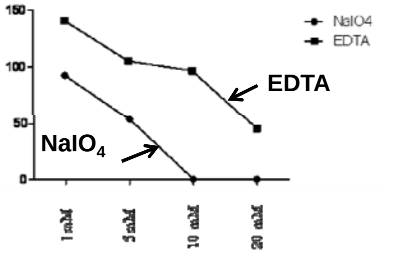
RVF VLP



RVFV binds via a charge-dependent binding to carbohydrate structures

RVF VLP binding was related to carbohydrate structures

% binding
A549 cells



RVF VLP binding was charge-dependent

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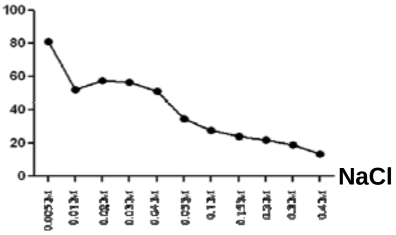
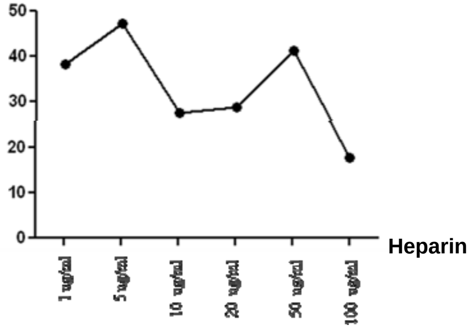


The cell surface carbohydrate attachment factor is linked to a protein

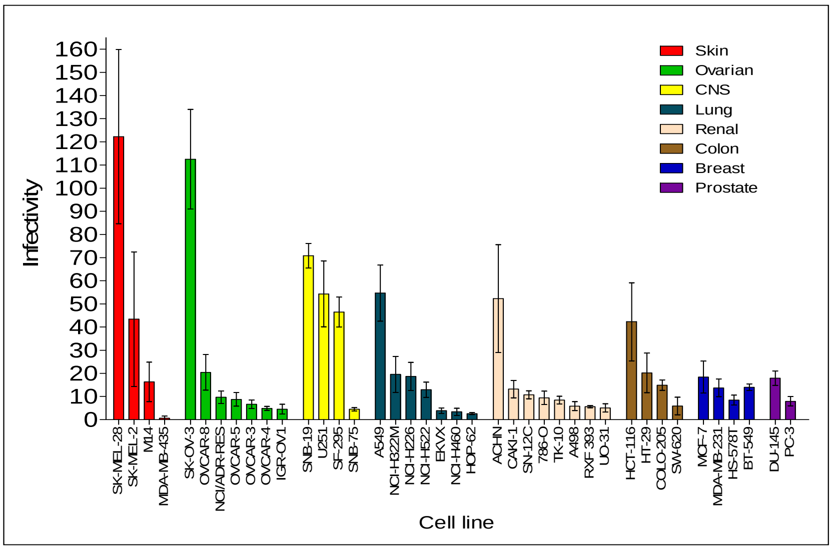
Cell treatment by trypsin and heparinase III partially reduced binding

Heparin – partial inhibition of binding

% binding
A549 cells



The structures involved in the entry of RVF VLPs are widely expressed – NCI60 cell library infection screen



Results presented as % luciferase signal compared with infected Vero-E6 cells

Selected genes positively associated with RVF VLP entry (microarray)

ILKAP	0,64	41	6,89E-06	Integrin-linked kinase-associated serine/threonine phosphatase 2C	
CCNA1	0,61	41	2,07E-05	Cyclin A1	
GJA3	0,61	41	2,52E-05	Gap junction protein, alpha 3, 46kDa	possible membrane protein
SORT1	0,6	41	3,30E-05	Sortilin 1	possible membrane protein
PRDM7	0,6	41	3,71E-05	PR domain containing 7	
SLC20A1	0,6	41	3,86E-05	Solute carrier family 20 (phosphate transporter), member 1	possible membrane protein
PPP1R14C	0,6	41	3,95E-05	Protein phosphatase 1, regulatory (inhibitor) subunit 14C	possible membrane protein
OCA2	0,59	41	4,17E-05	Oculocutaneous albinism II	possible membrane protein
MFAP3L	0,59	41	4,52E-05	Microfibrillar-associated protein 3-like	possible membrane protein
NT5DC1	0,59	41	4,72E-05	5'-nucleotidase domain containing 1	
BIRC7	0,59	41	4,95E-05	Baculoviral IAP repeat-containing 7 (livin)	
MAN1A2	0,59	41	5,39E-05	Mannosidase, alpha, class 1A, member 2	possible membrane protein
C22orf9	0,59	41	5,84E-05	Chromosome 22 open reading frame 9	
ZNF689	0,58	41	6,08E-05	Zinc finger protein 689	
RHOQ	0,58	41	6,15E-05	Ras homolog gene family, member Q	possible membrane protein
RGS1	0,58	41	7,54E-05	Regulator of G-protein signaling 1	possible membrane protein
TDRD3	0,58	41	8,03E-05	Tudor domain containing 3	
TMEM16D	0,58	41	8,37E-05	Transmembrane protein 16D	
LOC285000	0,57	41	8,78E-05	Hypothetical protein LOC285000	

Summary – viral entry

- The structures involved in the entry of RVF VLPs are widely expressed carbohydrate structures linked to proteins
- Investigation of pathways important for RVFV entry is underway

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Future RVFV

- Continued study of entry pathway
- Antiviral screening using VLPs or/and NSs del virus
- Prediction and prepardness against RVF outbreaks

New grant from SIDA 2012-2014 (Kenya, Sudan)

Other related studies

- Mosquito/arbovirus surveillance in Sweden

- Vector competence – applying for grants

Vector competence for RVFV viruses in Swedish mosquitoes

47 different blood-feeding mosquitoes in Sweden
Competent for RVFV transmission?
RVFV infection kinetics in mosquitoes

Virus surveillance in Swedish mosquitoes

Mosquito collection in Norr- and Västerbotten

- 15,959 mosquitoes collected 2009
- 15,301 *Aedes* species
- 9,963 *Aedes* communis

- *Culex, Culiseta, Coquilletidia, Anophel*es

Start-up of RNA preparation, virus detection

Prediction and prepardness against RVF outbreaks (in collaboration with Kenya, Sudan) SIDA 2012-2014

Aims

- Development of a risk-prediction model with high resolution
- Identification of factors that make specific geographic regions more prone to outbreaks.
- Identification of cultural and equality factors on appearance and consequences of outbreaks.
- Characterization of pastoralist mobility in a spatio-temporal framework.

Methods

Land-cover mapping and land-cover change analysis in relation to available geo-referenced health data using compiled climate and land-cover data

Human, animal and mosquito samples will be collected

Host-vector associations, genetic typing of mosquitoes, RVFV

Spatio-temporal analysis - nomadic herds in selected regions in Kenya will be tracked for a year by the use of GPS collars and automatic weather stations

- **Maria Baudin, PhD student, Umeå University**
- **Delowar Hossain, Masters student, Umeå University**
- **Clas Ahlm, Umeå University**
- **Göran Bucht, FOI, Umeå**
- **Jonas Näslund, FOI, Umeå**
- **Friedemann Weber, Marburg, Germany**
- **Rosemary Sang, icipe, Kenya**
- **Osama Ahmed Hassan, Sudan**

Rift Valley, Kenya 2010

Photo. Magnus Evander