How to diagnose Rift Valley Fever?

Catherine Cêtre-Sossah

« Control of exotic and emerging diseases»
CIRAD – BIOS

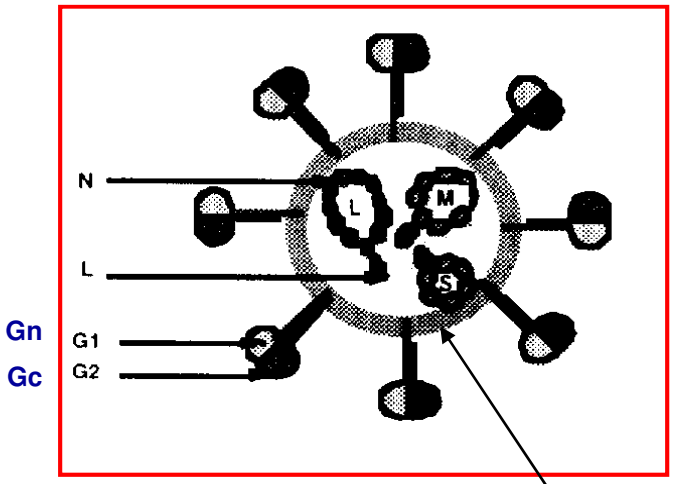
OIE Regional seminar on « RE-emergence of RVF in Southern Africa: How to better predict and respond?
Bloemfountain, South Africa, February 16-18th, 2009

Causal agent (1)

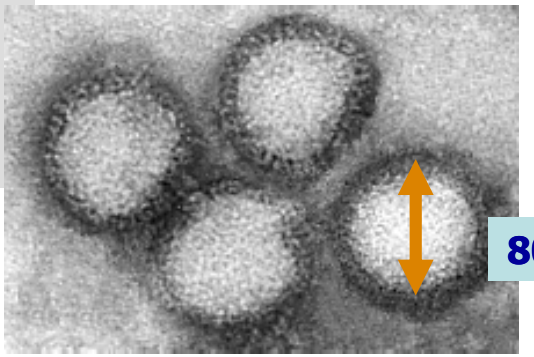
- Typical member of the
- Family: Bunyaviridae
- Genus: Phlebovirus

- 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



Bouloy M.



80-120 nm

Causal agent (2)

RNA dependant RNA polymerase

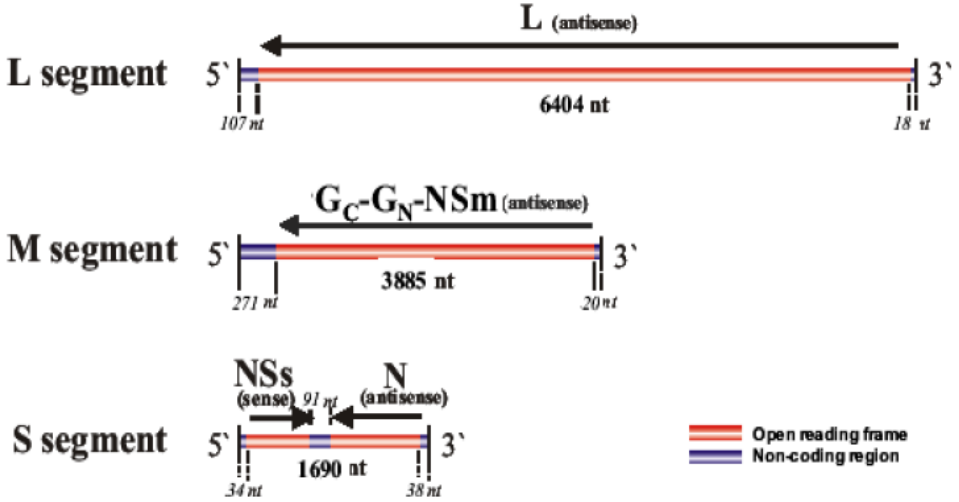


Figure 1. Schematic representation of the genome organization of RVFV MP12 strain. The overall segment length as well as the length of the flanking non-coding regions are indicated. L: viral RNA-dependent RNA polymerase, G_C: glycoprotein located at the C-terminus of the precursor molecule, G_N: glycoprotein located at the N-terminus of the precursor molecule, NSm: M segment-derived non-structural protein, NSs: S segment-derived non-structural protein.

Like all the Bunyaviridae, RVF has a tripartite single stranded RNA genome consisting of L(arge), M(edium) and S(mall) segments.

The L and M segments are of negative polarity and express respectively, the RNA dependant RNA polymerase L and 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

Similar to all negative stranded viruses, the RVFV genome is transcribed and replicated only when complexed with N and L, forming ribonucleoproteins (RNPs)

Causal agent and Phylogeny (3)

One single antigenic type (<5% of variation on nucleotides sequences M)

- **3 lineages or topotypes depending on the geographic origin Ia/ Central and East Africa , Ib/ West AFrica II/ Egypt/North AFrica**

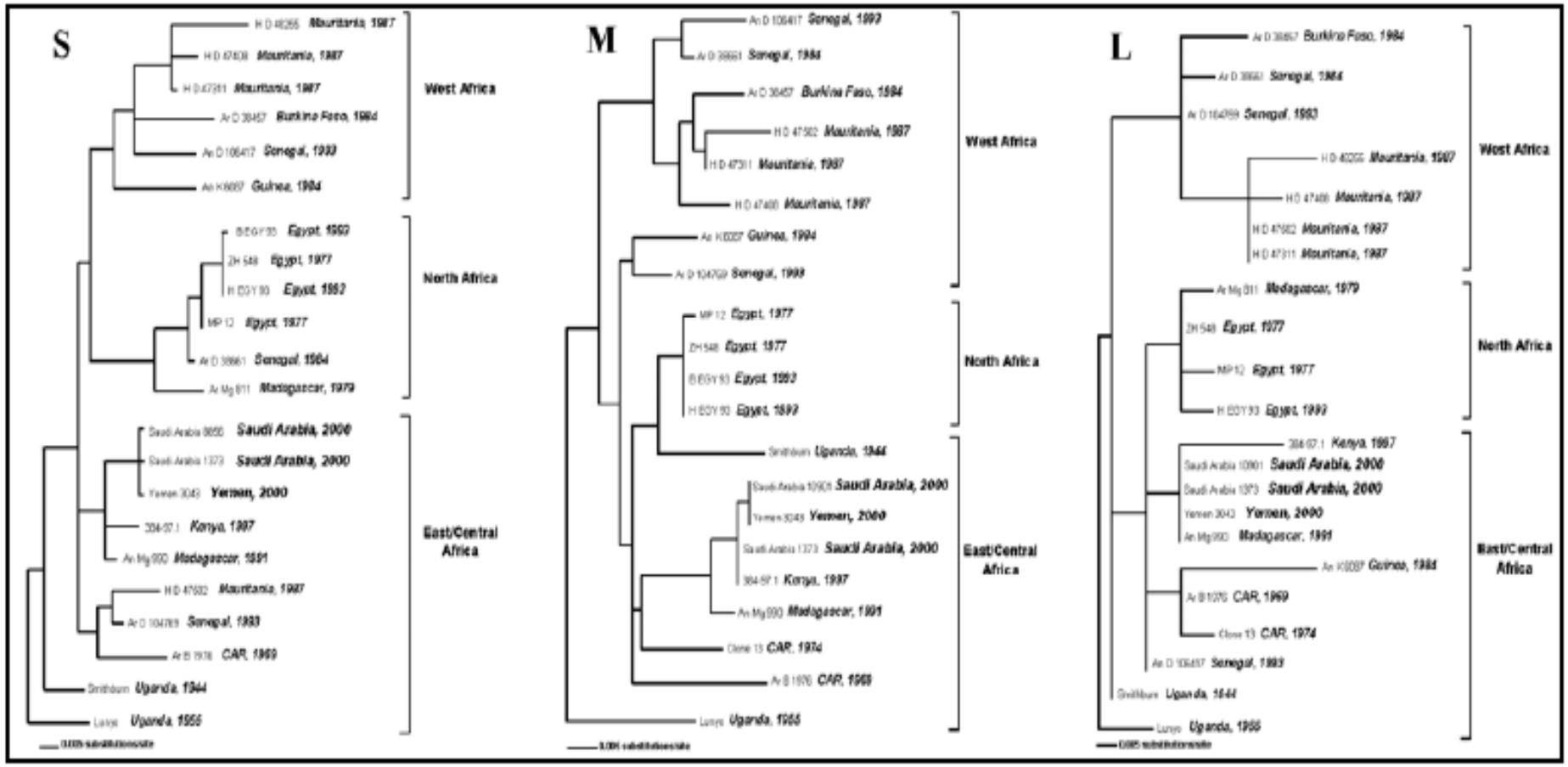


Figure. Phylogenetic relationship of the S, M, and L RNA segments of Rift Valley fever viruses. Maximum likelihood analysis of the nucleotide (nt) sequence differences among a 661-nt region of S RNA segment (Panel A), a 708-nt region of the M RNA segment (Panel B), and a 176-nt region of the L RNA segment (Panel C) of RVF viruses was performed by using PAUP4.0b10 (Sinauer Associates Inc., Sunderland, MA).

Schoemaker et al., 2002

Diagnosis in humans and animals

Field diagnosis, epidemiological, clinical and pathological features

Differential diagnosis

Laboratory diagnostic

- ✓ **Isolation and Identification of the agent (Culture, Histopathology, PCR)**
- ✓ **Serological tests (Virus neutralisation, Haemagglutination test, ELISA)**

Diagnosis in humans and animals

Field diagnosis, epidemiological, clinical and pathological features

RVF should be considered when there is a sudden onset of abortions at all stages of pregnancy (cattle, sheep and goats), sudden death in young animals following fever and obvious liver involvement in all cases. Variations occur with subclinical infections (transient viraemia with haemorrhages)

Differential diagnosis

- **Dengue, Chikungunya for humans**

- **Single cases of RVF can be confused with many viral diseases where there is sudden death, generalised lymphadenopathy and haemorrhages, Wesselsbron disease, Nairobi sheep disease** (mortality in old animals, sporadic, no hepatitis)**, Pasteurella multocida (haemorrages)**
- **Plant poisoning**
- **During abortions (brucellosis, leptospirosis, salmonellosis)**

Laboratory diagnosis

Samples needed

In case of Live animals

- **serum or blood collected in an anticoagulant (febrile stage of the disease)**

In case of dead animals

- **spleen, liver, kidney, lymph nodes, heart blood, brain of animals that have died, or from aborted fetuses**

Storage and Sending of the samples

Samples should be securely packed and labeled to indicate the dangerous nature of the contents, samples should be sent on ice at 4℃, or in formalin for histopathological studies if there is likely to be a delay

✓ **Isolation and Identification of the agent (Culture, Histopathology, PCR)**

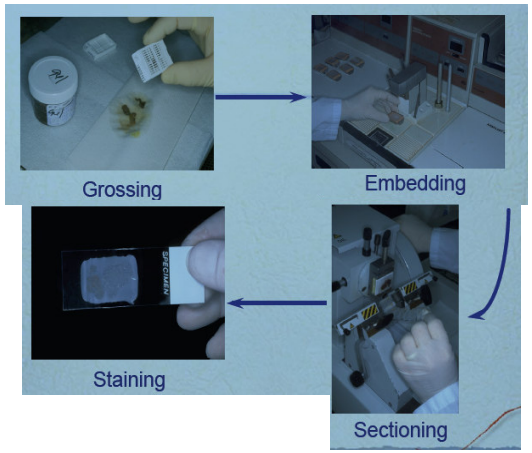
✓ **Serological tests (Virus neutralisation, ELISA, Haemagglutination Inhibition test-HAI)**

Identification of the agent (Culture, Histopathology, IFA)

Microscopy

- Electron microscopy (EM) (illustrate ultrastructure)
- confocal (increase morphologic dimension)

Histopathology



Samples needed

- **serum or blood collected in an anticoagulant (febrile stage of the disease)**
- **spleen, liver, kidney, lymph nodes, heart blood, brain of animals that have died, or from aborted fetuses**

IFA

Smears of liver spleen and brain followed by Immunofluorecence

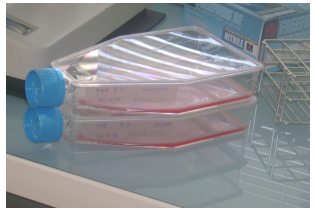
Virus isolation by Culture

Primary isolation usually performed in hamsters Intraperitoneal injections, in Infant 1 to 5 days old (Intracerebrally) or adult mice (intraperitoneally)

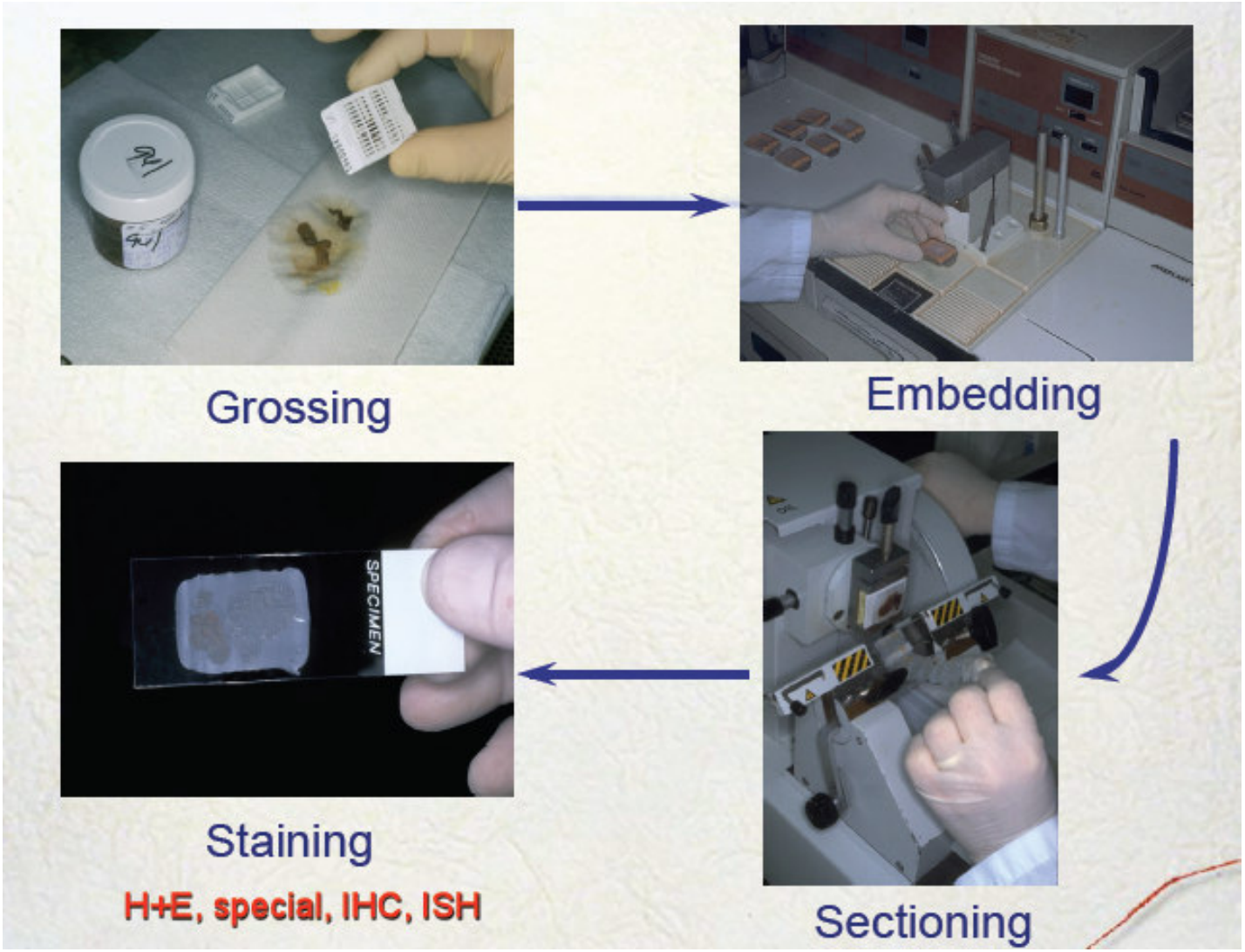
Death observed by day 2 (1 to 3)

Lambs, chicken eggs

Cell cultures of various types (Vero, BHK, CER (Chicken Embryo Reticulum) primary calves/lambs kidney or testis cells



Histopathological studies



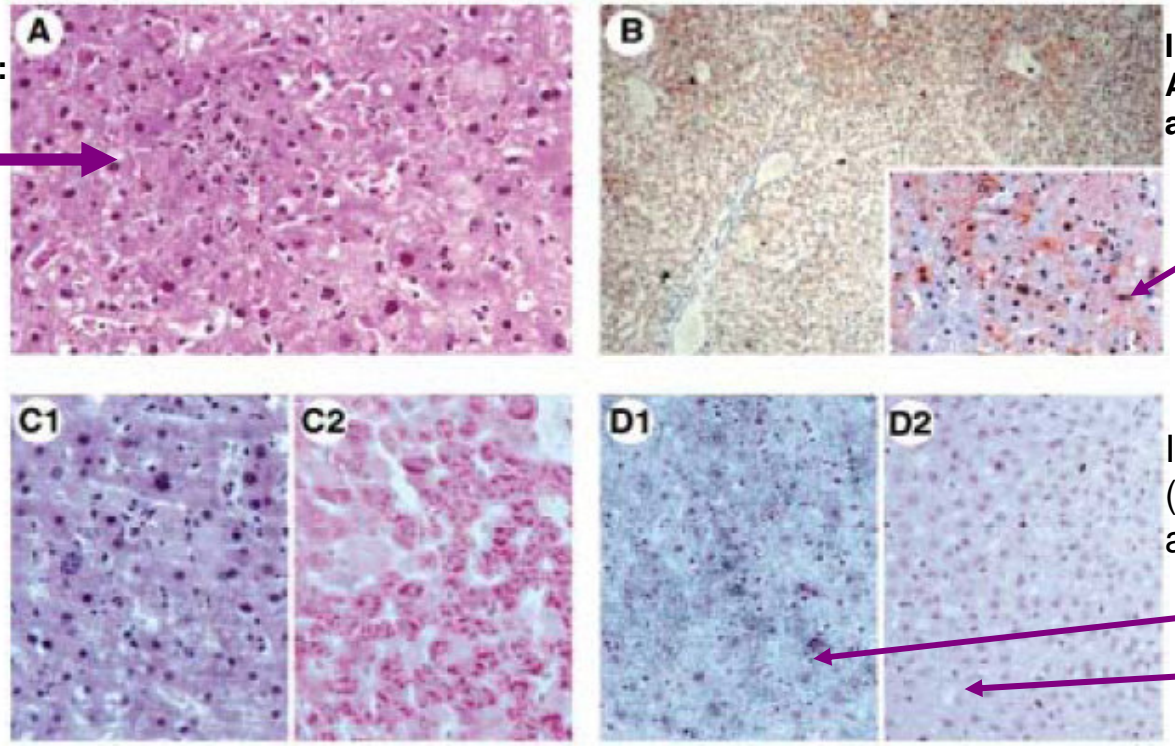
The antibodies used in IHC assays included a polyclonal rabbit anti-RVF virus antibody and a mouse anti-RVF virus antibody.

Identification of the agent (Histopathological studies)

Histopathological examination and immunohistochemical (IHC) assays

Hematoxylin/Eosin H/E): Show histopathological features

Also Histochemical stain (Gram, Silver): Highlight organisms



Immunohistochemicals Assay (IHC): localize antigens

Protéine N (segment S)

In situ hybridization (ISH): localize nucleic acids

Infected

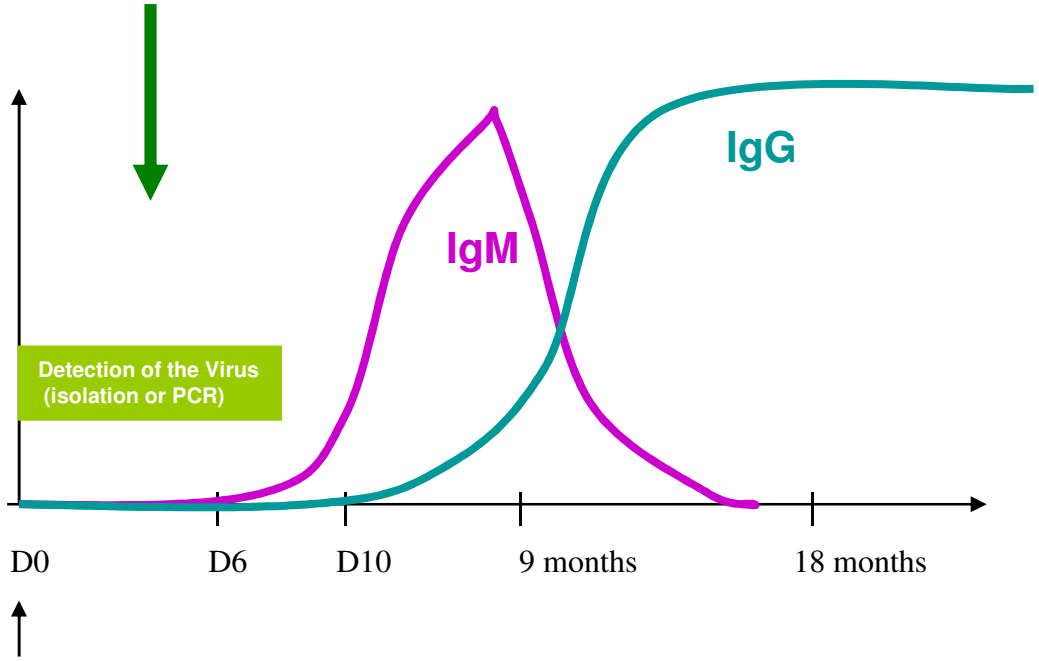
Uninfected

Figure 3. Fulminant hepatitis caused by attenuated Clone 13 in IFN-deficient mice. Histology, immunostaining, and in situ hybridization of post mortem liver sections from IFNAR2/2 mice inoculated with 10^4 PFU of Clone 13. (A) Hematoxylin-eosin staining showing perivascular coagulative necrosis and numerous apoptotic nuclei around the portal area. (B) Immunostaining for viral N protein. (C) Loss of glycogen as revealed by periodic acid Schiff staining. (D) In situ hybridization detecting virus-specific nucleic acids in infected (D1) or uninfected (D2) hepatocytes. Magnifications: (A and C) 3360; (B) 390; (inset) 3225; (D) 3225. (from Bouloy *et al.*, 2001).

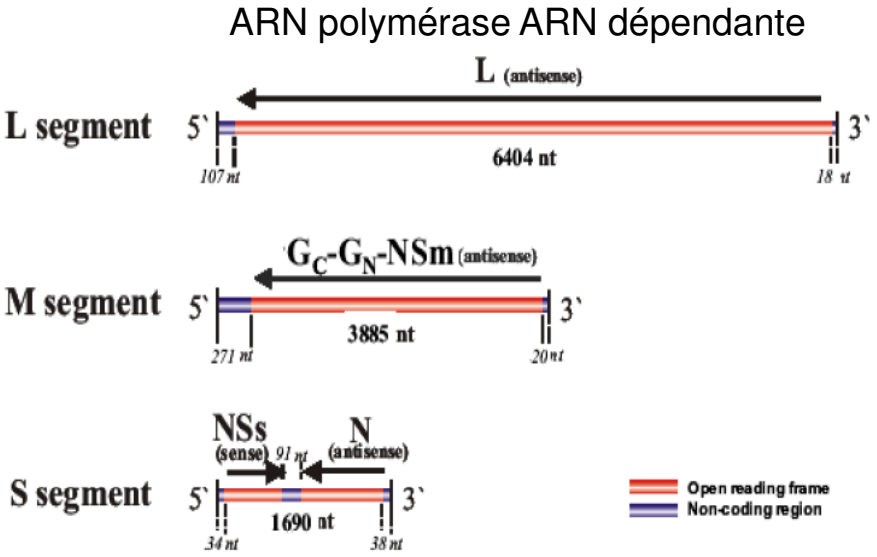
Advantages of Pathologic Methods

- Improves the speed, sensitivity, and specificity of microbial diagnosis
- Useful for identification of fastidious or slow-growing organisms organisms for which culture methods are unavailable or difficult
- Valuable for emerging infections whose causes are unknown
- Tests on fixed tissues minimizes biosafety concerns
- Fixed tissue samples allow retrospective studies

Identification of the agent (PCR)



Identification of the virus by genome amplification PCR (1)



L

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

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

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

Conventional PCR

✓ only one based on NSs

Real time PCR

✓ 6 Real time PCR developed and published on 4 different genes

Identification of the virus by PCR (2)

Realtime Reverse transcripton PCR with a Taqman probe based on L segment

Bird et al., 2007: Highly sensitive and broadly reactive QPCR assay

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2007, p. 3506–3513 Vol. 45, No. 11
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▽

Brian H. Bird,[1,2] Darcy A. Bawiec,[1] Thomas G. Ksiazek,[1] Trevor R. Shoemaker,[1] and Stuart T. Nichol[1]*

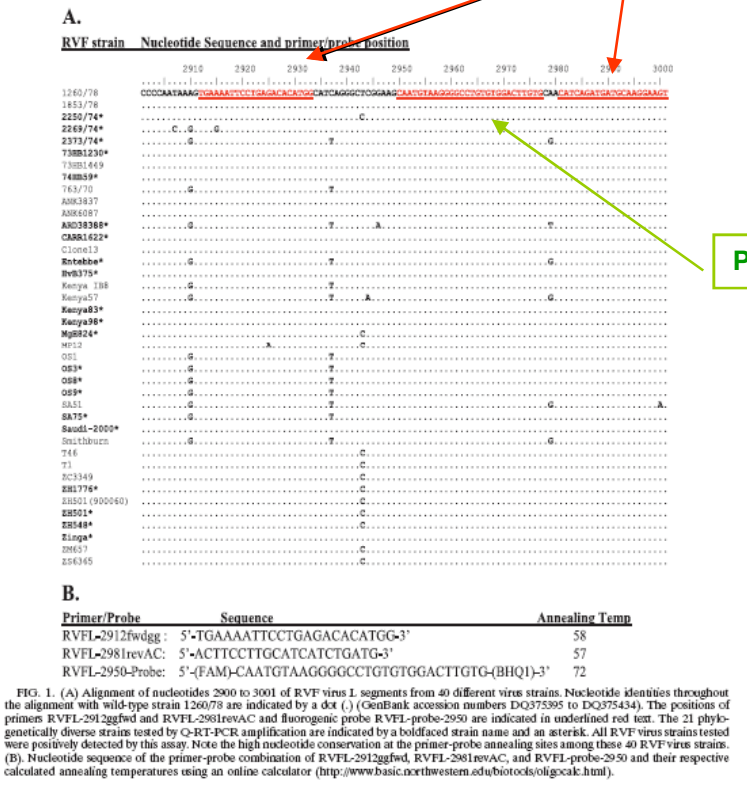
Special Pathogens Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, MS G-14, Atlanta, Georgia 30329,[1] and University of California, Davis, School of Veterinary Medicine, Davis, California 95616[2]

Received 4 May 2007/Returned for modification 11 July 2007/Accepted 13 August 2007

Rift Valley fever (RVF) virus is a mosquito-borne virus associated with large-scale epizootics/epidemics throughout Africa and the Arabian peninsula. Virus infection can result in economically disastrous "abortion storms" and high newborn mortality in livestock. Human infections result in a flu-like illness, with 1 to 2% of patients developing severe complications, including encephalitis or hemorrhagic fever with high fatality rates. There is a critical need for a highly sensitive and specific molecular diagnostic assay capable of detecting the natural genetic spectrum of RVF viruses. We report here the establishment of a pan-RVF virus quantitative real-time reverse transcription-PCR assay with high analytical sensitivity (~5 RNA copies of in vitro-transcribed RNA/reaction or ~0.1 PFU of infectious virus/reaction) and efficiency (standard curve slope = −3.66). Based on the alignments of the complete genome sequences of 40 ecologically and biologically diverse virus isolates collected over 56 years (1944 to 2000), the primer and probe annealing sites targeted in this assay are known to be located in highly conserved genomic regions. The performance of this assay relative to serologic assays is illustrated by testing of known RVF case materials obtained during the Saudi Arabia outbreak in 2000. Furthermore, analysis of acute-phase blood samples collected from human patients (25 nonfatal, 8 fatal) during that outbreak revealed that patient viremia at time of presentation at hospital may be a useful prognostic tool in determining patient outcome.

Primers

Probe



A.

RVF strain	Nucleotide Sequence and primer/probe position
1260/78	CCCCAATAAAGTGAAAATTCCTGAGACACATGGCATCAGGGCTCGGAAGCAATGTAAGGGGCCTGTGTGGACTTGTGCAACATCAGATGACAAGCAAGGAAGT
1853/78	
2250/74*	
2269/74*	
2373/74*	
73MB1230*	
73MB1449	
74MB59*	
763/70	
AN8387	
AN86087	
ARD38388*	
CAR81622*	
Clone13	
Entebbe*	
Hv8375*	
Kenya IBH	
Kenya57	
Kenya83*	
Kenya98*	
MgM824*	
MP12	
OS1	
OS3*	
OS8*	
OS9*	
SA51	
SA75*	
Saudi-2000*	
Smithburn	
746	
71	
SC3349	
ZH1776*	
ZH501 (900060)	
ZH501*	
ZH548*	
Zinga*	
ZM637	
ZS6365	

B.

Primer/Probe	Sequence	Annealing Temp
RVFL-2912fwdgg :	5'-TGAAAATTCCTGAGACACATGG-3'	58
RVFL-2981revAC:	5'-ACTTCCTTGCATCATCTGATG-3'	57
RVFL-2950-Probe:	5'-(FAM)-CAATGTAAGGGGCCTGTGTGGACTTGTG-(BHQ1)-3'	72

FIG. 1. (A) Alignment of nucleotides 2900 to 3001 of RVF virus L segments from 40 different virus strains. Nucleotide identities throughout the alignment with wild-type strain 1260/78 are indicated by a dot (.) (GenBank accession numbers DQ375395 to DQ375434). The positions of primers RVFL-2912ggfwd and RVFL-2981revAC and fluorogenic probe RVFL-probe-2950 are indicated in underlined red text. The 21 phylogenetically diverse strains tested by Q-RT-PCR amplification are indicated by a boldfaced strain name and an asterisk. All RVF virus strains tested were positively detected by this assay. Note the high nucleotide conservation at the primer-probe annealing sites among these 40 RVF virus strains. (B). Nucleotide sequence of the primer-probe combination of RVFL-2912ggfwd, RVFL-2981revAC, and RVFL-probe-2950 and their respective calculated annealing temperatures using an online calculator (http://www.basic.northwestern.edu/biotools/oligocalc.html).

40 aligned sequences
Detection of 5 copies of equivalent genome to 0,1 pfu

Identification du virus par PCR (3)

RT LAMP based on L segment
Peyrefitte et al., 2008: Real-time RT- LAMP for rapid detection of RVF

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2008, p. 000 Vol. 46, No. 11
0095-1137/08/$08.00+0 doi:10.1128/JCM.01188-08

Real-Time Reverse-Transcription Loop-Mediated Isothermal Amplification for Rapid Detection of Rift Valley Fever Virus▽

Christophe N. Peyrefitte,[1] Laetitia Boubis,[1] Daniel Coudrier,[2] Michèle Bouloy,[2] Marc Grandadam,[2] Hugues J. Tolou,[1] and Sébastien Plumet[1]*

Unité de virologie tropicale, Institut de Médecine tropicale du Service de Santé des Armées, BP 46, 13 998 Marseille armées, France,[1] and Unité de génétique moléculaire des Bunyavirus, Institut Pasteur, 25-28 rue du Dr Roux, 75724 Paris, Cedex 15, France[2]

Received 23 June 2008/Returned for modification 29 July 2008/Accepted 8 September 2008

The development and validation of a one-step, single-tube, real-time accelerated reverse-transcription loop-mediated isothermal amplification (RT-LAMP) for the detection of the L RNA segment of Rift Valley fever virus (RVFV) are described. The assay was performed at a constant temperature (63°C), with a real-time follow-up using a LightCycler and a double-stranded-DNA-intercalating fluorochrome. The assay is highly sensitive and comparable to real-time RT-PCR, with a detection limit of ~10 RNA copies per assay. However, the RT-LAMP assay is much faster than traditional RT-PCR and generates results in <30 min for most diluted samples. The specificity of the primers was established using other, related arboviruses as well as virus-containing and virus-free sera. The RT-LAMP assay reported here is thus a valuable tool for the rapid detection of RVFV in field diagnostic laboratories.

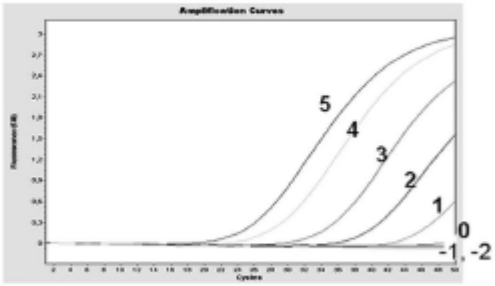
TABLE 3. Comparison of RVFV Taqman C_T and LAMP times for detection using real-time follow-up[a]

Estimated no. of RNA copies	C_T	
	Taqman RT-PCR (no. of cycles)	RT-LAMP (min)
100,000 (10^5)	18.2	7.9
10,000 (10^4)	20.3	8.5
1,000 (10^3)	27.4	10.5
100 (10^2)	33.4	12.4
10 (10^1)	39.9	18.0
~1 (10^0)	–	–
~0.1 (10^{-1})	–	–
~0.01 (10^{-2})	–	–

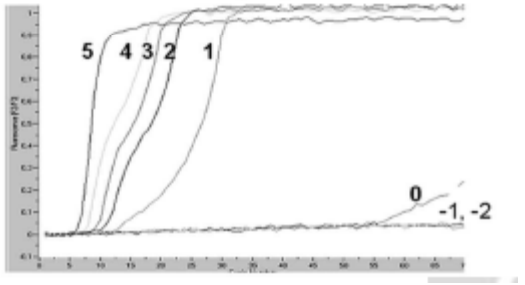
[a] RT-PCR Taqman and RT-LAMP were performed with a LightCycler instrument following procedures described in Materials and Methods. The C_T was determined by the fit points method at the end of the run. Note that for the Taqman PCR, the difference from C_T to C_T between dilutions is not 3, since efficiency of the reaction is not 2 (slope is −4 instead of −3,3) (8).

Real time follow up of amplifications

Taqman RT-QPCR FAM/TAMRA Light Cycler Channel F1



Real time RT LAMP BET Light cycler channel F2/F3



LAMP amplifies specific sequences on nucleic acids using a set of 6 primers and relies on the strand displacement activity on the DNA polymerase. RNA can be amplified simply by the addition of avian myeloblastosis virus RT to the reaction mix. High amplification rates lead to the production of dsDNA and of a white precipitate of magnesium pyrophosphate

Development of a one step, single tube, real time accelerated RT loop mediated isothermal amplification (RT LAMP)

- ✓**Constant temperature of 63°C with a light Cycler, a ds DNA SybrGreen**
- ✓**Sensitive, detection limit of 10 RNA copies**
- ✓**Faster than traditional RT PCR , generates results in < 30 minutes in most diluted samples**

Identification du virus par PCR (4)

Realtime Reverse transcripton PCR with a labeled Taqman Probe , based on the G2 (Gn) gene
Drosten et al., 2002: Rapid detection of RVF by RT PCR

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

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

Christian Drosten,* Stephan Göttig, Stefan Schilling, Marcel Asper, Marcus Panning, Herbert Schmitz, and Stephan Günther

Bernhard-Nocht-Institute of Tropical Medicine, Hamburg, Germany

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

Viral hemorrhagic fevers (VHFs) are acute infections with high case fatality rates. Important VHF agents are Ebola and Marburg viruses (MBGV/EBOV), Lassa virus (LASV), Crimean-Congo hemorrhagic fever virus (CCHFV), Rift Valley fever virus (RVFV), dengue virus (DENV), and yellow fever virus (YFV). VHFs are clinically difficult to diagnose and to distinguish; a rapid and reliable laboratory diagnosis is required in suspected cases. We have established six one-step, real-time reverse transcription-PCR assays for these pathogens based on the Superscript reverse transcriptase-Platinum *Taq* polymerase enzyme mixture. Novel primers and/or 5′-nuclease detection probes were designed for RVFV, DENV, YFV, and CCHFV by using the latest DNA database entries. PCR products were detected in real time on a LightCycler instrument by using 5′-nuclease technology (RVFV, DENV, and YFV) or SybrGreen dye intercalation (MBGV/EBOV, LASV, and CCHFV). The inhibitory effect of SybrGreen on reverse transcription was overcome by initial immobilization of the dye in the reaction capillaries. Universal cycling conditions for SybrGreen and 5′-nuclease probe detection were established. Thus, up to three assays could be performed in parallel, facilitating rapid testing for several pathogens. All assays were thoroughly optimized and validated in terms of analytical sensitivity by using in vitro-transcribed RNA. The ≥95% detection limits as determined by probit regression analysis ranged from 1,545 to 2,835 viral genome equivalents/ml of serum (8.6 to 16 RNA copies per assay). The suitability of the assays was exemplified by detection and quantification of viral RNA in serum samples of VHF patients.

RVFV

	RVS	RVP	RVAs (rc)	
oligos	AAAGGAACAATGGACTCTGGTCA	AAAGCTTTGATATCTCTCAGTGCCCCAA	ATTGGAGGACATGGTAGTAAGAAGTG	
				n of 43
NC_002044	aaaggaacaatggactctggtca/	aaagctttgatatctctcagtgccccaa/	attggaggacatggtagtaagaagtg	12
M33074	...../	...../	.....*	14
AF134495	...../	...../	.....c.....	3
AF134503	...../	...../	.....g.....	3
AF334492	.....t...../	.....t...../	.....c.....	2
M33094	.....g...../	....t...../	.....*	2
AF134499	.....a...../	...../	.....	1
AF134502	.....a.t...../	...a...../	.....	1
AF134497	...../	.....t..../	.....c.....	1
AF134500	...../.g...../	...../	.....	1
M33073	.....c...../	...../	.....	1
M33095	..g....t...../	...../	.....*	1
M33080	...../.g...../	...../	.....g.....*	1

13 sequences aligned

Detection of 2, 835 virus equivalent genome per ml (2, 143 à 1, 525)

Using qRTPCR test to rapidly identify highly viremic RVF case
Njenga MK, Paweska J, Wanjala R, Drosten C, Breiman R. et al. JCM, 2009

Level of viremia in fatal cases were significantly higher than dose in non fatal cases (mean $10^{5.2}$ verus $10^{2.0}$ per ml
The Case Fatality ratio (CFR) was 50% among cases with a Ct value<27 and 4.5% among cases with Ct>27 giving

- **a sensitivity of 93.8% and a 95.5% for the negative predictive value**
- **a specificity of 58% and a 50% of positive predictive value**

Identification du virus par PCR (5)

SybrGreen Realtime Reverse transcripton PCR, the only one developed on the N gene

Naslund et al., 2008: Kinetics of RVF in experimentally infected mice

Journal of Virological Methods xxx (2008) xxx–xxx

Contents lists available at ScienceDirect

Journal of Virological Methods

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

Kinetics of Rift Valley Fever Virus in experimentally infected mice using quantitative real-time RT-PCR

Jonas Näslund [a,b,c], Nina Lagerqvist [a,b,d], Åke Lundkvist [d], Magnus Evander [c], Clas Ahlm [b], Göran Bucht [a,e,*]

[a] Swedish Defence Research Agency, Department of CBRN Defence and Security, SE-901 82 Umeå, Sweden
[b] Division of Infectious Diseases, Department of Clinical Microbiology Umeå University, SE-901 85 Umeå, Sweden
[c] Division of Virology, Department of Clinical Microbiology Umeå University, SE-901 85 Umeå, Sweden
[d] Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden
[e] National Environment Agency, Environmental Health Institute, 11 Biopolis Way, 06-05/08, Helios Block, Singapore 138667, Singapore

ARTICLE INFO

Article history:
Received 12 November 2007
Received in revised form 31 March 2008
Accepted 3 April 2008
Available online xxx

Keywords:
Real-time PCR
Rift Valley Fever Virus
Animal model
Kinetics

ABSTRACT

Rift Valley Fever (RVF) is an important viral zoonosis in Africa affecting animals and humans. Since no protective vaccines or effective treatments are available for human use, accurate and reliable diagnostic methods are essential for surveillance of the disease in order to implement adequate public health actions. To study the kinetics of the RVF Virus (RVFV) infection, a SYBR Green-based quantitative real-time RT-PCR assay was developed. By using primers targeting the S-segment of RVFV, the detection limit of this assay was estimated to 30 RNA templates. Blood and organs of experimentally infected mice were sampled at different time points and RVFV RNA was quantified. High amounts of RVFV RNA were found in blood, brain, and liver samples shortly after infection with a 1–4 days post infection window for viral RNA detection. Mice developed symptoms after the appearance of serum antibodies, indicating that the host response plays an important role in the outcome of the disease. The RVFV quantitative RT-PCR proved to be a valuable diagnostic tool during the first days of infection, before detectable antibody levels and visual symptoms of RVF were observed.

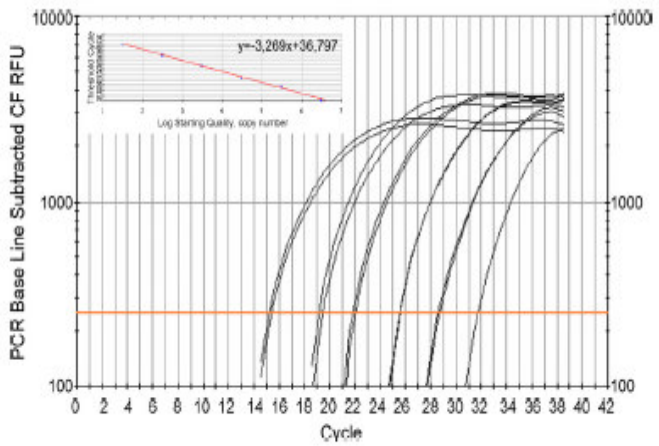


Fig. 2. QRT-PCR performed on serial dilutions of *in vitro* transcribed RNA standards. C_T values plotted against log transformations of the 10-fold serial diluted RNA standard are depicted. The resulting equation of the standard curve was $y = -3.269x+36.797$ and the assay displayed linearity between 3×10 and 3×10^6 copies. The intra- and inter-assay coefficient of variation based on C_T values was calculated to be 0.1–2.9% and 2.5–3.5%, respectively.

30 RNA Templates

Identification du virus par PCR (6)

Nested conventional Reverse Transcription PCR based on the NSs gene (Segment S)

- 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

ELSEVIER

Journal of Virological Methods 91 (2001) 85–92

Journal of Virological Methods

www.elsevier.com/locate/jviromet

Single-tube and nested reverse transcriptase–polymerase chain reaction for detection of Rift Valley fever virus in human and animal sera

A.A. Sall [a], J. Thonnon [a], O.K. Sene [a], A. Fall [a], M. Ndiaye [a], B. Baudez [a], C. Mathiot [a], M. Bouloy [b,*]

[a] *Institut Pasteur de Dakar, BP 220 Dakar, Senegal*
[b] *Groupe des Bunyavirides, Institut Pasteur, 28, Rue du Dr Roux, 75724 Paris Cedex 15, France*

Received 25 May 2000; received in revised form 16 August 2000; accepted 18 August 2000

Abstract

Rift Valley fever (RVF) is an anthropozoonosis caused by a *Phlebovirus* (Bunyaviridae family) that has re-emerged recently in East and West Africa in 1997–1998. This emphasizes the need for early and rapid detection of the virus and an efficient surveillance system. To this goal, a single tube or a nested reverse transcriptase–polymerase chain reaction (RT–PCR) method focusing on the NSs coding region of the S segment was developed and used to detect the RVF virus (RVFV) genome, resulting respectively in the synthesis of 810 and 662 bp DNA amplimers. The assay was specific for RVFV and did not amplify any other phleboviruses known to circulate in sub-Saharan Africa. When serial dilutions of RVFV were artificially mixed with human normal serum, the minimal detection limits were 50 and 0.5 plaque forming units respectively using the simple and the nested RT–PCR. The RT–PCR method was efficient for the detection of RVFV RNA in the blood from experimentally RVFV-infected mice and lamb and the nested RT–PCR was found more sensitive than the virus isolation method. Additionally, this detection method was applied successfully for the diagnosis of human cases during the 1998 Mauritanian outbreak.

Keywords: Bunyaviridae; Diagnosis; *Phlebovirus*

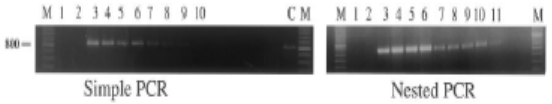


Fig. 2. Detection limits of RVF viral RNA in human sera using simple (A) or nested RT–PCR (B). (A) 10^7 pfu (lane 3), 10^6 pfu (lane 4) 10^5 pfu (lane 5), 10^4 pfu (lane 6), 10^3 pfu (lane 7), 10^2 pfu (lane 8), 50 pfu (lane 9), 10 pfu, (lane 10), C: positive control. (B) 10^7 pfu (lane 3), 10^5 pfu (lane 4),10^3 pfu (lane 5), 5×10^2 pfu (lane 6), 10^2 pfu (lane 7), 50 pfu (lane 8), 10 pfu (lane 9), 5 pfu (lane 10), 0.5 pfu (lane 11). Uninfected controls (lanes1 and 2), M: marker

Table 2
Detection of virus and viral genome in the serum of experimentally RVFV-infected mice

	Surviving mice	Virus isolation		Simple RT–PCR		Nested RT–PCR	
		Positive/tested	%	Positive/tested	%	Positive/tested	%
Day 0	25	0/25	0	0/25	0	0/25	0
Day 1	25	4/25	16	2/25	8	18/25	72
Day 2	23	16/23	70	1/23	4.3	23/23	100
Day 3	17	12/17	70	3/17	17.6	13/17	76
Day 4	7	3/7	43	2/7	28.5	4/7	57
Day 5	3	0/3	0	0/3	0	0/3	0

Detection from D1 to D4, nested PCR technique is more sensitive than the one step one but contaminations are easy to get- Detection of 100 of RNA copies

Sensitivity of 70,6 % (12 out of 17 échantillons)
Specificity of 97,1 % (268 out of 276 échantillons)

Identification du virus par PCR (7)

Realtime Reverse transcription PCR with a Taqman probe
Garcia et al., 2001: Quantitative realtime PCR for the detection of RVF

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

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]*

Unité de Virologie, Centre de Recherches du Service de Santé des Armées (CRSSA) Emile Pardé, Grenoble,[1] and Groupe des Bunyaviridés, Institut Pasteur, Paris,[2] France

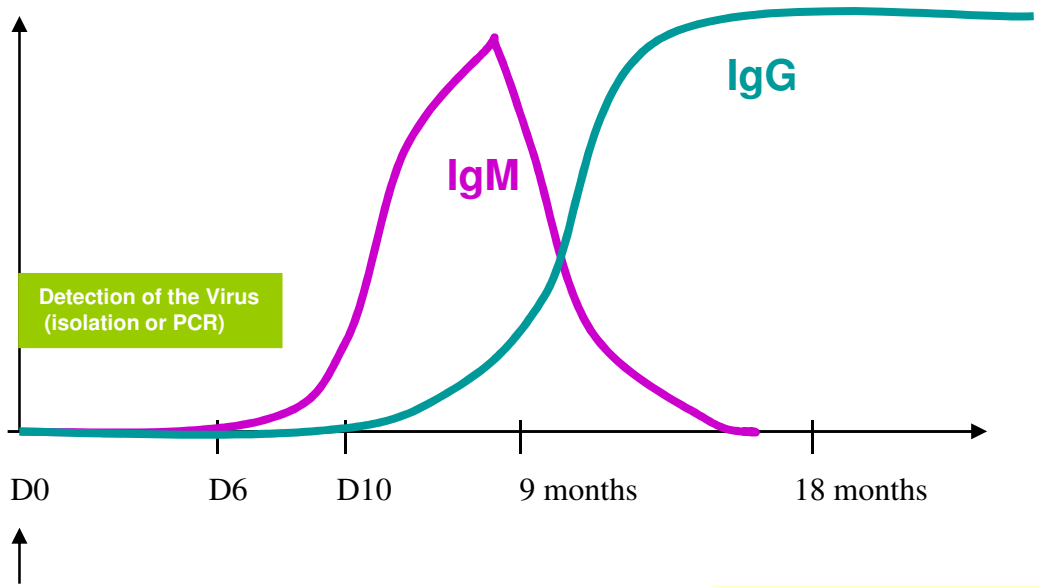
Received 6 August 2001/Returned for modification 12 September 2001/Accepted 27 September 2001

The Rift Valley fever virus (RVFV), a member of the genus *Phlebovirus* (family *Bunyaviridae*) is an enveloped negative-strand RNA virus with a tripartite genome. Until 2000, RVFV circulation was limited to the African continent, but the recent deadly outbreak in the Arabian Peninsula dramatically illustrated the need for rapid diagnostic methods, effective treatments, and prophylaxis. A method for quantifying the small RNA segment by a real-time detection reverse transcription (RT)-PCR using TaqMan technology and targeting the nonstructural protein-coding region was developed, and primers and a probe were designed. After optimization of the amplification reaction and establishment of a calibration curve with synthetic RNA transcribed in vitro from a plasmid containing the gene of interest, real-time RT-PCR was assessed with samples consisting of RVFV from infected Vero cells. The method was found to be specific for RVFV, and it was successfully applied to the detection of the RVFV genome in animal sera infected with RVFV as well as to the assessment of the efficiency of various drugs (ribavirin, alpha interferon, 6-azauridine, and glycyrrhizin) for antiviral activity. Altogether, the results indicated a strong correlation between the infectious virus titer and the amount of viral genome assayed by real time RT-PCR. This novel method could be of great interest for the rapid diagnosis and screening of new antiviral compounds, as it is sensitive and time saving and does not require manipulation of infectious material.

Detection of 100 of RNA copies, as the conventional nested PCR

Specific antibody detection

Specific anti RVF Kinetics IgM or IgG



Virus neutralisation, HAI (Haemagglutination inhibition)
Indirect Immunofluorescence (IFA)
ELISA
AGID, Complement fixation

Identification of the agent

Microscopy
Needs the equipment

Drawbacks/ Advantages

IFA

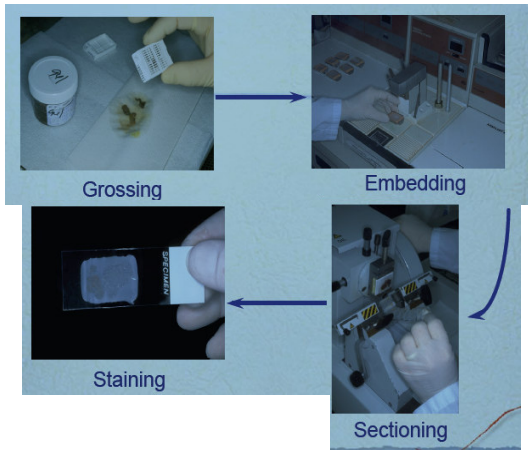
Smears of liver spleen and brain

Quick, sensitive, alternative technique

PCR

Quick, sensitive, Different protocols
Limits: Possibilities for sequencing parts of the genome

Histopathology



- Speed, sensitivity, specificity
- Useful for identification for viruses fo which culture is not available in the lab
- Use of fixed tissues, minimizes biosafety concerns
- Fixed tissues samples allow retrospective studies

Virus isolation by Culture

Possibilities for sequencing the full viral genome
With isolation

Primary isolation on primary calves/lambs kidney or testis cells very quick, does not need
Cell lines culture,

Chicken eggs, quick

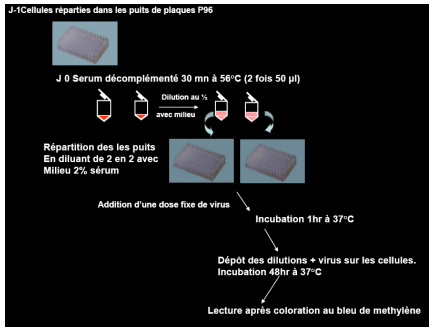
Cell cultures of various types (Vero, BHK, CER (Chicken Embryo Reticulum)
expensive to maintain

Specific antibody detection (Virus neutralisation, HAI, ELISA, AGID, C^T fixation)

➢ **Virus neutralisation**

- Neutralisation test (plaque reduction), requires cell culture equipped laboratory working with the live virus (enzootic countries), but very specific test and can be used early in the infection

- Haemagglutination inhibition test (HAI) can give cross reactivity with other members of the Phleboviruses, not commonly used nowadays



➢ **ELISA**

-Indirect ELISA –species specific-

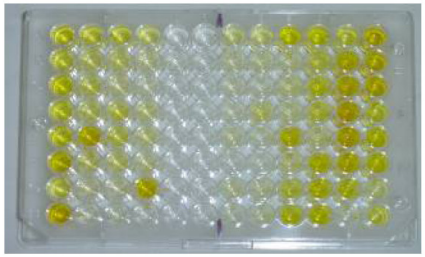
-with an inactivated antigen – IgG and IgM -Se more than 99% for small ruminants and 92% for bovines-Spe more than 99% (Paweska, 2003)

-or the N recombinant protein (Fafetine, 2007, Jansen Van Vuren, 2007, Paweska, 2008)

-Competitive ones:- all species- IgM type or IgG type (home made by different institutes such as Pasteur Institute, CDC), or commercialised

- Kit IgG, competitive IgG Kit working on different species (humans, with a very high sensitivity depending on the species from 99, 47 to 100 %), with a high specificity (99,29 to 100 % depending on the species)

-Kit IgM, with high specificity of 97,4 to 99,4%and sensitivity of 100 %



➢**AGID and complement fixation have been used in the past**

How to work with this virus

- ✓ Enveloped Virus, can be inactivated by formol, beta propriolactone (1/1000), hypochlorite de sodium (residual chlore >5ppm)
- ✓ stable Virus with a pH between 6,2 and 8, inactivation with a pH <6,2
- ✓ Heat stability: temperature of 56°C for 3 hr to inactivate
- ✓ Stable several months at 4°C in blood an sera, 1 month at -20°C

Biosafety level 3 laboratory or cabinet for

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

Gloves, Mask


Thanks for your attention