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HAL Id: hal-01274555
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Submitted on 22 Mar 2018

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Development of real-time RT-PCR for the detection of low concentrations of Rift Valley fever virus

Marianne Maquart\textsuperscript{a,b,c}, Sarah Temmam\textsuperscript{c,d}, Jean-Michel Héraud\textsuperscript{e}, Isabelle Leparc-Goffart\textsuperscript{f}, Catherine Cêtre-Sossah\textsuperscript{a,b,c}, Koussay Dellagi\textsuperscript{c,g}, Eric Cardinale\textsuperscript{a,b,c}, Hervé Pascalis\textsuperscript{c,g}

Author affiliations: \textsuperscript{a} CIRAD, UMR CMAEE, F-97490 Sainte-Clotilde, La Réunion, France; \textsuperscript{b} INRA, UMR 1309 CMAEE, F-34398 Montpellier, France; \textsuperscript{c} CRVOI, F-97490 Sainte Clotilde, La Réunion, France; \textsuperscript{d} CNRS-Université Claude Bernard- UMR 5557, F-69000 Lyon, France; \textsuperscript{e} Institut Pasteur de Madagascar, Virology Unit, Antananarivo, Madagascar; \textsuperscript{f} IRBA antenne Marseille, Centre National de Référence (CNR) des Arbovirus, F-13007 Marseille, France; \textsuperscript{g} IRD, F-97490 Sainte Clotilde, La Réunion, France

Corresponding author: Marianne Maquart, CIRAD, UMR 15 CMAEE CRVOI Plateforme de recherche CYROI, 2 rue Maxime Rivière, F-97490 Sainte Clotilde, La Réunion, France
Tel.: +262 6 92 435588
E-mail address: mariannemaquart@yahoo.fr
Abstract (193 words)

In recent years, Madagascar and the Comoros archipelago have been affected by epidemics of Rift Valley fever (RVF), however detection of Rift Valley fever virus (RVFV) in zebu, sheep and goats during the post epidemic periods was frequently unsuccessful. Thus, a highly sensitive real-time RT-PCR assay was developed for the detection of RVFV at low viral loads. A new RVF SYBR Green RT-PCR targeting the M segment was tested on serum from different RVF seronegative ruminant species collected from May 2010 to August 2011 in Madagascar and the Comoros archipelago and compared with a RVF specific quantitative real time RT-PCR technique, which is considered as the reference technique. The specificity was tested on a wide range of arboviruses or other viruses giving RVF similar clinical signs. A total of 38 out of 2,756 serum samples tested positive with the new RT-PCR, whereas the reference technique only detected 5 out of the 2,756. The described RT-PCR is an efficient diagnostic tool for the investigation of enzootic circulation of the RVF virus. It allows the detection of low viral RNA loads adapted for the investigations of reservoirs or specific epidemiological situations such as inter-epizootic periods.

Keywords: Rift Valley fever virus; diagnostic tool; semi-nested RT-PCR; inter-epidemic period
1. Introduction

Rift Valley fever (RVF) is an arthropod-borne zoonotic disease affecting mainly domestic and wild ruminants as well as humans (Olive et al., 2012). Rift Valley fever virus (RVFV) is an enveloped RNA segmented virus belonging to the family Bunyaviridae, genus Phlebovirus, transmitted by a wide range of mosquito species with a tri-segmented single-stranded RNA genome of negative or ambisense polarity (S, M and L) (Suzich et al., 1990; Müller et al., 1994; Nichol et al., 2001; Schmaljohn and Hopper, 2001; Bird et al., 2007a; Turrell et al., 2008). Outbreaks have been reported in 19 countries from continental Africa, Indian Ocean islands (Madagascar, Union of the Comoros, Mayotte) and the Arabian Peninsula (Balenghien et al., 2013). Epidemiology of RVF is complex, associated with specific ecologic and climatic conditions such as heavy rainfalls that favor the emergence of a large number of adult mosquitoes maintaining RVF during interepidemic periods (IEP) through vertical transmission in Aedes vectors for Southern and Eastern Africa (Linthicum et al., 1985; Swanepoel and Coetzer, 2004). In addition, RVF isolations have been successful in forest-sampled mosquitoes in the Afro-Malagasy area, implicating wild vertebrates as potential reservoir hosts (Fontenille, 1989; Smithburn et al., 1948).

Whether the emergence of outbreaks in insular ecosystems is due to enzootic maintenance of the virus within the island or to importation from continental landmasses is unknown in the majority of cases (Tortosa et al., 2012). Recently, Bird et al. (2008) confirmed that RVFV activity can be detected at low levels in animal reservoirs during IEPs. In 2007, human RVF cases were noticed in 2 islands of the Comoros archipelago, Mayotte and Grande Comore (Sissoko et al., 2009).

Even if no case has been reported in this area since, seroconversion in animals has been observed in Madagascar and the Comoros archipelago including Mayotte, indicating persisting viral circulation in a post-epidemic period (Roger et al., 2009; Cêtre-Sossah et al.,
2012). Considering the proximity of these islands and the continuous animal trade between
them, an active surveillance system for RVF was implemented between May 2010 and
August 2011. The surveillance network targeting farm animal species was specifically
designed to pick up active virus circulation in the context of the current post-epidemic phase
in Madagascar and the Comoros archipelago. The implementation of a highly sensitive and
specific diagnostic tool was a prerequisite for this large survey.

Techniques for the diagnosis of RVF include virus isolation, detection of specific antibody
responses, and detection of the viral nucleic acids. Enzyme-linked immunosorbent assays,
based on antigens or the recombinant nucleocapsid protein of RVFV have been extensively
validated for the serodiagnosis of RVF (World Organisation for Animal Health, 2004;
Paweska et al., 2005, 2008). **Reverse transcriptase PCR (RT-PCR)** assays are currently the
most rapid and sensitive tests for the detection and quantification of RVFV. Conventional RT-
PCR (Sall et al., 2002) and real-time RT-PCR (rtRT-PCR) based on TaqMan probe
technology are the most used techniques during outbreaks (Garcia et al., 2001; Drosten et al.,
2002; Bird et al., 2007b; Weidmann et al., 2008). Several studies have highlighted the highest
analytical sensitivity obtained when using two-steps protocol RT-PCR detection (Sall et al.,
2002). In order to improve the detection of very low concentrations of viral nucleic acids in
samples taken during a RVF post-epidemic or inter-epizootic period, a highly sensitive,
RVFV specific, real-time semi-nested RT-PCR detection system was developed. Specificity
and sensitivity of the test was comparatively assessed with one of the currently available
techniques used as reference. The new RT-PCR proved to be more adapted to detect low-level
of RVF circulation in specific epidemiological conditions such as those in Comoros
archipelago, Mayotte and Madagascar where seroconversions occur in the absence of clinical
signs, and is therefore a useful tool for the study of RVF circulation during IEPs.
The main objective was to develop a highly sensitive real-time RT-PCR assay for the detection of Rift Valley fever virus.

2. Material and Methods

2.1. Cells, Viruses and samples

Vero cells were grown at 37°C in Dulbecco’s modified medium (DMEM) (GIBCO, USA) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% non essential amino-acids, 1% fungizone, 1% penicillin-streptomycin.

The following RVFV strains were used: the Smithburn vaccine strain obtained from the OBP (Ondersterpoort biological products, Onderstepoort, South Africa), four distinct Mauritanian RVF strains isolated during the 2010 outbreak (MT24010, MT25010, MT26010, MT28010) by the LNERV/ISRA laboratories respectively in Mauritania and Senegal and three RVF strains from Madagascar isolated during the 2008-2009 outbreak (776-08, 1464-08, 1585-08) by the Institut Pasteur, Madagascar.

The animal cohort (204 zebu in Madagascar, 104 zebu, sheep and goats in Mayotte and 182 zebu, sheep and goats in the Union of Comoros) was chosen because of their RVF seronegative status tested with a commercial ELISA kit (Comtet et al., 2010). The animals were bled monthly as part of an epidemiological survey held in a post-epidemic period from May 2010 to August 2011, giving a total of 2,756 serum samples. Serum samples were stored at -80°C.

2.2. RNA extraction

Viral RNA was extracted from 10 µl of either Smithburn strain infected Vero cell supernatant titrating to $10^{6.3}$ TCID$_{50}$/ml or from 200 µl of a pool of 5 serum samples and spiked with an internal control MS2 bacteriophage (Ninove et al., 2011) by using the EZ1 Virus Mini kit.
v2.0 (Qiagen, Hilden, Germany) and the EZ1 robot, according to the manufacturer’s protocol.

Positive pools were analyzed individually with the aim of identifying the positive individual.

2.3. Primer design

The complete mRNA segments of 52 RVF virus isolates retrieved from GenBank were aligned via Muscle (Edgar, 2004) using the Geneious pro software package (v5.5) (Drummond et al., 2011). A highly conserved 110 bp genomic region within the RVF Gc-glycoprotein gene was identified matching 100% of the selected RVF isolates (nucleotides 2,656 to 2,766). This region was used for the design of the forward and reverse primers.

2.4. cDNA synthesis and real-time PCR conditions

cDNA templates were produced from a 20 µl RNA aliquot with 0.4 µg of random hexamers (Promega, Madison, USA) by reverse transcription using the GoScript kit (Promega, Madison, USA) in a final volume of 40 µl, as described by the manufacturer. The 2,756 samples collected during the epidemiological serosurvey were also analyzed without the step of reverse transcription to verify the absence of residual DNA.

The following primers listed in figure 1 were determined to be optimal: RVFV-M-2,656F, RVFV-M-2,840R (first round PCR) and RVFV-M-2,766R (second round semi-nested PCR).

Several experiments highlighted that the highest analytical sensitivity could be obtained using a two-step RT-PCR protocol detection. To avoid cross-contamination, experiments were performed within an imposed one-way laboratory environment where amplified PCR products were never manipulated in the same area as the original samples. Additionally, individual tubes were used and multiple negative controls and no positive control were included in each PCR protocol. Amplification reaction mixtures were prepared in a final volume of 25 µl consisting of 12.5 µl GoTaq qPCR SYBR Green mastermix 2X (Promega, Madison, USA) and 5 µl of the nucleic acid matrix. For the first round PCR, 0.1 µM of each primer RVFV-M-2,656F and RVFV-M-2,840R was used while 0.4 µM of each primer, RVFV-M-2,656F and
RVFV-M-2,766R was used for the second round PCR. Thermal cycling for both amplification steps was performed in a Mx3005P real-time thermocycler (Stratagene, USA) as follows:

95°C for 5 min; 45 cycles of [95°C for 15 s, 60°C for 30 s and 72°C for 45 s]. A dissociation analysis was carried out following PCR to identify the right product by its specific melting temperature. The dissociation cycle was 95°C for 1 min, 60°C for 30 s and heating to 95°C at 0.1°C/s with continuous measurement of fluorescence. The expected sizes of these PCR products are 185 bp and 110 bp for the first and second round PCR, respectively.

2.5. Comparative efficiency

The performance of the new RT-PCR was compared with that of a Taqman RT-PCR considered as the current reference detection system (Bird et al., 2007b; Escadafal et al., 2013). The comparison was made by determining the detection limit of each assay using 10-fold serial dilutions (10^{-2} to 10^{-9}), prepared from the supernatant of Vero cells that had previously been infected by the Smithburn strain. The supernatant titrated to 10^{6.3} TCID_{50}/ml.

2.6. Analytical specificity of the new RT-PCR

RNAs from other vector-borne viruses were tested, as well as other viruses giving clinical signs similar to RVF, such as abortions or fever. These viruses are listed in Table 1.

2.7. Analytical sensitivity based on field samples

The sensitivity of the new RT-PCR was assessed on a bank of 2,756 serums collected from 490 zebu, sheep and goats that were serially bled in Madagascar and the Comoros archipelago and compared to the same Taqman RT-PCR (Bird et al., 2007b; Escadafal et al., 2013) and calibrated using a standard control based on the dilution range described above. Samples tested positive with the new RT-PCR were considered as true positives only after sequencing of the amplified material.

2.8. Serological status of the RVF RT-PCR samples
The serological status of the 490 animals was assessed monthly with a commercial ELISA kit (Comtet et al., 2010).

2.9. Sequence analysis

PCR products sequencing was performed by Beckman’s Coulter Genomics, UK. Blast alignments were generated using MUSCLE through the Geneious pro software package (v5.5) (Biomatters Ltd, UK) (Edgar, 2004; Drummond et al., 2011).

2.10. Statistical analysis

Data were recorded and analyzed statistically with the R software package (version 3.0.1) (R Development Core team, 2009). Results with a two-sided p-value ≤ 0.05 were scored as being significant.

3. Results

3.1. Primer design

From the analysis of multiple sequence alignments of the M segment of 52 biologically and geographically diverse RVF virus strains, a highly conserved region was identified at positions 2511 to 2880 as illustrated in figure 1. Three distinct primers spanning this region were designed.

The nucleotide alignment and the position of the three primers are presented in Figure 1: two of the primers (RVFV-M-2656F, RVFV-M-2766R) gave a 100% match with all strains and the remaining primer named RVFV-M-2840R included 2 degenerated nucleotides (Fig. 1).

3.2. Comparative efficiency

Based on ten-fold serial dilutions of the Smithburn vaccine strain, the reference Taqman RT-PCR test (Bird et al., 2007b; Escadafal et al., 2013) was able to detect RVFV to a dilution of $10^{-3}$ whereas the new RT-PCR was able to detect to a dilution of $10^{-5}$ (Fig 2).

3.3. Analytical specificity
No false positive or false negative results were observed: All viruses belonging to different tested viral families (listed in Table 1) were negative using the new RT-PCR, and all PCR products obtained during screening gave the expected RVFV sequence in sequence analysis.

3.4. Analytical sensitivity based on field samples

All serum samples from the 2,756 serially bled zebu, sheep and goats were tested by the new RT-PCR and by the reference technique. A total of five samples tested positive by the reference RT-PCR, whereas 38 samples tested positive using the new RT-PCR, including all five detected using the reference technique. The new RT-PCR system, thus, demonstrated at least a 7.7-fold ($p=2.313\times10^{-7}$) higher sensitivity than the reference system (Table 2). Integration of RNA viruses into host genomes has previously been demonstrated (Katzourakis and Gifford, 2010), however, no PCR products were amplified in the absence of reverse transcription, thus excluding this as a possibility for tested RVFV PCR-positive animals.

3.5. Serological status of the RVF RT-PCR samples

Serum samples were available for consecutive months of sampling for 18 of the 38 RT-PCR positive animals. Seven of these animals seroconverted one month after sampling, whereas 11 animals did not (Table 3).

4. Discussion

Highly sensitive molecular assays have already been developed for the detection of RVFV based either on conventional PCR or on real-time PCR. These techniques have been shown to diagnose RVFV infection during epidemics. The specifically low viral loads associated with post-epidemic or inter-epidemic periods require extreme sensitivity and robustness from detection systems for the successful study of RVF circulation in potential reservoir animals during these epidemiological phases.
The presented RT-PCR proved to be both specific and sensitive and could detect $2 \log$ dilutions further than the reference Taqman system (Bird et al., 2007b; Escadafal et al., 2013). Using 2,756 serum samples collected in the two countries as a test group, the reference technique detected only 5 positive samples. In contrast, the new RT-PCR allowed detection of 38 positive samples, corresponding to at least a 7.7 times higher sensitivity than the currently available gold-standard.

The new RT-PCR detection system performed well, even in the presence of mutations. On the forward-sense primer RVFV-M-2656F, 100% of homology was observed among all strains and on the antisense primer RVFV-M-2766R, only one mutation was observed on the first nucleotide for 6 strains. Genomic diversity was assessed and showed the presence of 7 mutations in 2 sequences (CVII 14 and CVII 15) and 4 mutations for 2 sequences (ABMR 030 and ABMR 040), all isolated from Anjouan (Figure 3). The remaining sequences only presented erratic mutations. These results confirmed the strength of the primer designed in 2 conserved areas.

In 2007, the first cases of RVF were reported in the Comoros archipelago with evidence of animal seroconversions. During the epidemiological survey conducted between May 2010 and August 2011, the incidence of infection assessed by animal seroconversion was estimated at 14.4% in the Union of Comoros in goats, sheep and zebu and 4.94% in Madagascar in zebu. Out of the 18 positive animals detected by the new RT-PCR, 7 animals seroconverted with a specific RVF IgG antibody response, 11 did not seroconvert. The fact that a large proportion of animals that tested PCR positive did not show evidence of seroconversion to RVFV as assessed by Elisa is interesting and deserves comments. Firstly, the serology-based detection methods employed have inherent limits to the level of antibody that they can detect. More generally, the scale of the host immune response mounted after an infection is dependant on several host-associated factors including age, health, nutritional status and
immuno competence of the host. The pathogen infecting dose and its potential to actively
replicate in dendritic cells are also likely to correlate with the magnitude of the invoked
immune response, and therefore low-level responses may be expected during inter-epidemic
periods, when viral loads are (on the whole) low. During epizootics, occasional animals may
prove positive to the pathogen by molecular techniques but fail to show evidence of
seroconversion or have delayed seroconversion. In a serosurvey of naturally acquired caprine
arthritis encephalitis virus infection in goats, almost 20% of seronegative animals and
apparently healthy animals had positive PCR test results and only half of them seroconverted
by 8 months later (Rimstad et al., 1993). All these factors are likely operational in the field
conditions characteristic of Madagascar and Comoros and may have implications to the
epidemiology of RVFV; particularly, whether animals detected PCR positive but failed to
seroconvert are contagious and participate to the low level transmission of RVF in the post
epidemic period is presently unknown. The new RT-PCR allowed the detection of RVFV in
38 samples collected in a global epidemiological network in the Comoros archipelago and
Madagascar considered as insular ecosystems (Tortosa et al., 2012). Both serological and
molecular findings strongly suggest that the RVF virus is circulating in this area without
inducing any clinical signs in infected animals.

During IEPs, the epidemiology of RVF remains unclear and involves a possible cryptic
enzootic transmission cycle (Linthicum et al., 1985; Bird et al., 2008). Up to now, the
detection of the RVF virus in RVF potential species reservoirs such as bats (Roussetus
obliviosus, Chaerephon pusillus, Miniopterus griveaudi) in Comoros (n=96) and Maki catta
(Lemur catta) in Mayotte (n=52) was unsuccessful (unpublished data), as well as in
Madagascar (Olive et al.; 2013). The question of a possible inside virus propagation via an
animal reservoir or a transitory animal host in the Indian Ocean area is still addressed. The
use of sensitive detection tools will be a great help in the understanding of the RVF inter-
epidemic circulation.

Acknowledgments
The authors would like to thank the CRVOI team, La Réunion (Drs Roger and Girard, C.
Foray) for their implication in the epidemiological survey. They also thank Dr D.A.
Wilkinson for his comments and careful reading, all the field team in Madagascar (J.
Rasolofoniaina, N. Andriamanampy, M.-M. Olive) the veterinarians (Drs Evenar, Rabenja,
Remoro) and veterinarian assistants of Tulear I, Tulear II, Mampikony districts, the FOFIFA-
DRZV (Drs J. Ravaomanana and R. Rakotondravao), Dr L. Bibias Armand and the Comorian
partners (Mohamed Halifa) for their assistance during the collection campaign and P. Gil, for
her help in lab experiments at CIRAD, Montpellier.

References
Balenghien, T., Cardinale, E., Elissa, N., Failloux, A.-B., Nipomichene, T.N.J.J., Nicolas, G.,
Rakotoharinome, V.M., Roger, M., Zumbo, B., 2013. Towards a better understanding of Rift
genome analysis of 33 ecologically and biologically diverse Rift Valley fever virus strains
reveals widespread virus movement and low genetic diversity due to recent common ancestry.
J. Virol. 81, 2805-2016.
sensitive and broadly reactive quantitative reverse transcription-PCR assay for high-


Figures captions

Figure 1

(A) Alignment of nucleotides 2653 to 2850 of M segment from 52 strains from different origins. Identical nucleotides for all strains are represented by a dot. Strains are identified by their country of origin and date of isolation. Primers were represented on the consensus sequence of the alignment and designed from the most conserved parts of the alignment. Only the RVFV-M-2840R primer was degenerated for 2 nucleotides. (B) Nucleotide sequences, position on the M segment and annealing temperatures of the primers (Tm calculator http://www6.appliedbiosystems.com/support/techtools/calc/).

Figure 2

Comparative efficiency of the Taqman RT-PCR and the new RT-PCR of ten-fold serial dilutions of tissue culture infected supernatant starting from a Smithburn strain stock titrating to $10^{6.3}$ TCID$_{50}$/ml. Dilutions detected with (A) the Taqman RT-PCR (Bird et al., 2007) (B) the new RT-PCR.

Figure 3

Alignment of nucleotides 2653 to 2766 of M segment from 52 strains of different origin and from 30 strains detected with the new RT-PCR. Identical nucleotides for all strains are represented by a dot. Strains are identified by their country of origin and date of isolation.

Anj: Anjouan, GC: Grande Comore, Mg: Madagascar, Mo: Moheli
## Table 1
List of viruses tested with the newly developed RT-PCR detection system

<table>
<thead>
<tr>
<th>Genus</th>
<th>Species</th>
<th>Vector-borne virus</th>
<th>RVF syndrome like associated</th>
<th>Main host</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phlebovirus</strong></td>
<td>Belterra virus</td>
<td><em>Culicidae</em></td>
<td>-</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Icoaraci virus</td>
<td><em>Culicidae</em></td>
<td>-</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Punta Toro virus</td>
<td><em>Culicidae</em></td>
<td>-</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Toscani virus</td>
<td><em>Phlebotominae</em></td>
<td>Fever</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Sandfly Naples virus</td>
<td><em>Phlebotominae</em></td>
<td>-</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td>Sandfly Sicilia virus</td>
<td><em>Phlebotominae</em></td>
<td>-</td>
<td>Human</td>
</tr>
<tr>
<td><strong>Flavivirus</strong></td>
<td>West Nile virus</td>
<td><em>Culicidae</em></td>
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<td>Human/Animal</td>
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<td>Dengue type 1 virus</td>
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<td>Fever</td>
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<tr>
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<td>Dengue type 3 virus</td>
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<td>Fever</td>
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<td>Japanese encephalitis virus</td>
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<td>Apoï virus</td>
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<td>-</td>
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<td>Chikungunya virus</td>
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<td>O’Nyong nyong virus</td>
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<td>Schmallenberg virus</td>
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<td><strong>Reovirus</strong></td>
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<tr>
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<td>H5N1 virus A</td>
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</table>

NA: Not Applicable
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<thead>
<tr>
<th>Collection area</th>
<th>Number of animals collected during the serosurvey</th>
<th>New RT-PCR, based on the M segment* (% of positive animals)</th>
<th>Taqman RT-PCR (Bird et al., 2007), based on the L segment (% of positive animals)</th>
<th>Incidence levels of seroconversions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayotte</td>
<td>104</td>
<td>3/124 (0.29)</td>
<td>0/124 (0)</td>
<td>ND</td>
</tr>
<tr>
<td>Union of Comoros</td>
<td>182</td>
<td>20/1063 (10.9)</td>
<td>3/1063 (1.6)</td>
<td>14.4</td>
</tr>
<tr>
<td>Madagascar</td>
<td>204</td>
<td>15/1569 (7.35)</td>
<td>2/1569 (0.9)</td>
<td>4.94</td>
</tr>
</tbody>
</table>

*Each of the positive PCR sample detected with the new RT-PCR on M segment was confirmed by sequence analyses. ND: Not determined
Table 3
Serological status of the 38 RT-PCR positive samples

<table>
<thead>
<tr>
<th></th>
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<td>-</td>
<td>+</td>
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<td>+*</td>
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*Samples detected by the Taqman RT-PCR (Bird et al., 2007)
B. Primer | Sequence | Nucleotide position on the M segment | Annealing T
--- | --- | --- | ---
RVFV-M-2656F | 5'-CTAGCCGTTCACAAACTGGG-3' | 2,656 - 2,676 | 60
RVFV-M-2840R | 5'-GACTGARGAYTCTGAATTGCACC-3' | 2,817 - 2,840 | 55
RVFV-M-2766R | 5'-CAATTGCATACCCTTTGCCTGGGC-3' | 2,742 - 2,766 | 70

Figure 1
Figure 2

A.

B.
Highlights

- A highly sensitive real-time PCR assay for the detection of Rift Valley Fever virus
- Sensibility tested on 2,756 serum from Madagascar and the Comoros archipelago
- Comparison with a RVF specific quantitative real time RT-PCR reference technique
- System specific and 7.7 times more sensitive than the reference technique
- Diagnostic tool for the detection of low viral loads in inter-epizootic periods