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

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1 **Abstract (193 words)**

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

16

17 **Keywords:** Rift Valley fever virus; diagnostic tool; semi-nested RT-PCR; inter-epidemic
18 period

1 **1. Introduction**

2 **Rift Valley fever** (RVF) is an arthropod-borne zoonotic disease affecting mainly domestic and
3 wild ruminants as well as humans (Olive et al., 2012). Rift Valley fever virus (RVFV) is an
4 enveloped RNA segmented virus belonging to the family *Bunyaviridae*, genus *Phlebovirus*,
5 transmitted by a wide range of mosquito species **with a tri-segmented single-stranded RNA**
6 **genome of negative or ambisense polarity** (S, M and L) (Suzich et al., 1990; Müller et al.,
7 1994; Nichol et al., 2001; Schmaljohn and Hopper, 2001; Bird et al., 2007a; Turell et al.,
8 2008). **Outbreaks have been reported in 19 countries from continental Africa, Indian Ocean**
9 **islands (Madagascar, Union of the Comoros, Mayotte) and the Arabian Peninsula (Balenghien**
10 **et al., 2013).** Epidemiology of RVF is complex, associated with specific ecologic and climatic
11 conditions such as heavy rainfalls that favor the emergence of a large number of adult
12 mosquitoes maintaining RVF during interepidemic periods (IEP) through vertical
13 transmission in *Aedes* vectors for Southern and Eastern Africa (Linthicum et al., 1985;
14 Swanepoel and Coetzer, 2004). In addition, RVF isolations have been successful in forest-
15 sampled mosquitoes in the Afro-Malagasy area, implicating wild vertebrates as potential
16 reservoir hosts (Fontenille, 1989; Smithburn et al., 1948).

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

23 Even if no case has been reported in this area since, seroconversion in animals has been
24 observed in Madagascar and the Comoros archipelago including Mayotte, indicating
25 persisting viral circulation in a post-epidemic period (Roger et al., 2009; Cêtre-Sossah et al.,

1 2012). Considering the proximity of these islands and the continuous animal trade between
2 them, an active surveillance system for RVF was implemented between May 2010 and
3 August 2011. The surveillance network targeting farm animal species was specifically
4 designed to pick up active virus circulation in the context of the current post-epidemic phase
5 in Madagascar and the Comoros archipelago. The implementation of a highly sensitive and
6 specific diagnostic tool was a prerequisite for this large survey.

7 Techniques for the diagnosis of RVF include virus isolation, detection of specific antibody
8 responses, and detection of the viral nucleic acids. Enzyme-linked immunosorbent assays,
9 based on antigens or the recombinant nucleocapsid protein of RVFV have been extensively
10 validated for the serodiagnosis of RVF (World Organisation for Animal Health, 2004;
11 Paweska et al., 2005, 2008). Reverse transcriptase PCR (RT-PCR) assays are currently the
12 most rapid and sensitive tests for the detection and quantification of RVFV. Conventional RT-
13 PCR (Sall et al., 2002) and real-time RT-PCR (rtRT-PCR) based on TaqMan probe
14 technology are the most used techniques during outbreaks (Garcia et al., 2001; Drosten et al.,
15 2002; Bird et al., 2007b; Weidmann et al., 2008). Several studies have highlighted the highest
16 analytical sensitivity obtained when using two-steps protocol RT-PCR detection (Sall et al.,
17 2002). In order to improve the detection of very low concentrations of viral nucleic acids in
18 samples taken during a RVF post-epidemic or inter-epizootic period, a highly sensitive,
19 RVFV specific, real-time semi-nested RT-PCR detection system was developed. Specificity
20 and sensitivity of the test was comparatively assessed with one of the currently available
21 techniques used as reference. The new RT-PCR proved to be more adapted to detect low-level
22 of RVF circulation in specific epidemiological conditions such as those in Comoros
23 archipelago, Mayotte and Madagascar where seroconversions occur in the absence of clinical
24 signs, and is therefore a useful tool for the study of RVF circulation during IEPs.

1 The main objective was to develop a highly sensitive real-time RT-PCR assay for the
2 detection of Rift Valley fever virus.

3

4 **2. Material and Methods**

5 *2.1. Cells, Viruses and samples*

6 Vero cells were grown at 37°C in Dulbecco's modified medium (DMEM) (GIBCO, USA)
7 containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1% non essential amino-acids,
8 1% fungizone, 1% penicilin-streptomycin.

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

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

21 *2.2. RNA extraction*

22 Viral RNA was extracted from 10 µl of either Smithburn strain infected Vero cell supernatant
23 titrating to 10^{6.3} TCID₅₀/ml or from 200 µl of a pool of 5 serum samples and spiked with an
24 internal control MS2 bacteriophage **(Ninove et al., 2011)** by using the EZ1 Virus Mini kit

1 v2.0 (Qiagen, Hilden, Germany) and the EZ1 robot, according to the manufacturer's protocol.
2 Positive pools were analyzed individually with the aim of identifying the positive individual.

3 2.3. Primer design

4 The complete M RNA segments of 52 RVF virus isolates retrieved from GenBank were
5 aligned via Muscle (Edgar, 2004) using the Geneious pro software package (v5.5)
6 (Drummond et al., 2011). A highly conserved 110 bp genomic region within the RVF Gc-
7 glycoprotein gene was identified matching 100% of the selected RVF isolates (nucleotides
8 2,656 to 2,766). This region was used for the design of the forward and reverse primers.

9 2.4. cDNA synthesis and real-time PCR conditions

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

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

17 Several experiments highlighted that the highest analytical sensitivity could be obtained using
18 a two-step RT-PCR protocol detection. To avoid cross-contamination, experiments were
19 performed within an imposed one-way laboratory environment where amplified PCR products
20 were never manipulated in the same area as the original samples. Additionally, individual
21 tubes were used and multiple negative controls and no positive control were included in each
22 PCR protocol. Amplification reaction mixtures were prepared in a final volume of 25 µl

23 consisting of 12.5 µl GoTaq qPCR SYBR Green mastermix 2X (Promega, Madison, USA)
24 and 5 µl of the nucleic acid matrix. For the first round PCR, 0.1 µM of each primer RVFV-M-
25 2,656F and RVFV-M-2,840R was used while 0.4 µM of each primer, RVFV-M-2,656F and

1 RVFV-M-2,766R was used for the second round PCR. Thermal cycling for both amplification
2 steps was performed in a Mx3005P real-time thermocycler (Stratagene, USA) as follows:
3 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
4 analysis was carried out following PCR to identify the right product by its specific melting
5 temperature. The dissociation cycle was 95°C for 1 min, 60°C for 30 s and heating to 95°C at
6 0.1°C/s with continuous measurement of fluorescence. The expected sizes of these PCR
7 products are 185 bp and 110 bp for the first and second round PCR, respectively.

8 *2.5. Comparative efficiency*

9 The performance of the new RT-PCR was compared with that of a Taqman RT-PCR
10 considered as the current reference detection system (Bird et al., 2007b; Escadafal et al.,
11 2013). The comparison was made by determining the detection limit of each assay using 10-
12 fold serial dilutions (10^{-2} to 10^{-9}), prepared from the supernatant of Vero cells that had
13 previously been infected by the Smithburn strain. The supernatant titrated to $10^{6.3}$ TCID₅₀/ml.

14 *2.6. Analytical specificity of the new RT-PCR*

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

17 *2.7. Analytical sensitivity based on field samples*

18 The sensitivity of the new RT-PCR was assessed on a bank of 2,756 serums collected from
19 490 zebu, sheep and goats that were serially bled in Madagascar and the Comoros archipelago
20 and compared to the same Taqman RT-PCR (Bird et al., 2007b; Escadafal et al., 2013) and
21 calibrated using a standard control based on the dilution range described above. Samples
22 tested positive with the new RT-PCR were considered as true positives only after sequencing
23 of the amplified material.

24 *2.8. Serological status of the RVF RT-PCR samples*

1 The serological status of the 490 animals was assessed monthly with a commercial ELISA kit
2 (Comtet et al., 2010).

3 *2.9. Sequence analysis*

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

7 *2.10. Statistical analysis*

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

11

12 **3. Results**

13 *3.1. Primer design*

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

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

21 *3.2. Comparative efficiency*

22 Based on ten-fold serial dilutions of the Smithburn vaccine strain, the reference Taqman RT-
23 PCR test (Bird et al., 2007b; Escadafal et al., 2013) was able to detect RVFV to a dilution of
24 10^{-3} whereas the new RT-PCR was able to detect to a dilution of 10^{-5} (Fig 2).

25 *3.3. Analytical specificity*

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

4 *3.4. Analytical sensitivity based on field samples*

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

13 *3.5. Serological status of the RVF RT-PCR samples*

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

18 **4. Discussion**

19 Highly sensitive molecular assays have already been developed for the detection of RVFV
20 based either on conventional PCR or on real-time PCR. These techniques have been shown to
21 diagnose RVFV infection during epidemics. The specifically low viral loads associated with
22 post-epidemic or inter-epidemic periods require extreme sensitivity and robustness from
23 detection systems for the successful study of RVF circulation in potential reservoir animals
24 during these epidemiological phases.

1 The presented RT-PCR proved to be both specific and sensitive and could detect 2 log
2 dilutions further than the reference Taqman system (Bird et al., 2007b; Escadafal et al., 2013).
3 Using 2,756 serum samples collected in the two countries as a test group, the reference
4 technique detected only 5 positive samples. In contrast, the new RT-PCR allowed detection of
5 38 positive samples, corresponding to at least a 7.7 times higher sensitivity than the currently
6 available gold-standard.

7 The new RT-PCR detection system performed well, even in the presence of mutations. On the
8 forward-sense primer RVFV-M-2656F, 100 % of homology was observed among all strains
9 and on the antisense primer RVFV-M-2766R, only one mutation was observed on the first
10 nucleotide for 6 strains. Genomic diversity was assessed and showed the presence of 7
11 mutations in 2 sequences (CVII 14 and CVII 15) and 4 mutations for 2 sequences (ABMR
12 030 and ABMR 040), all isolated from Anjouan (Figure 3). The remaining sequences only
13 presented erratic mutations. These results confirmed the strength of the primer designed in 2
14 conserved areas.

15 In 2007, the first cases of RVF were reported in the Comoros archipelago with evidence of
16 animal seroconversions. During the epidemiological survey conducted between May 2010
17 and August 2011, the incidence of infection assessed by animal seroconversion was estimated
18 at 14.4 % in the Union of Comoros in goats, sheep and zebu and 4.94 % in Madagascar in
19 zebu. Out of the 18 positive animals detected by the new RT-PCR, 7 animals seroconverted
20 with a specific RVF IgG antibody response, 11 did not seroconvert. The fact that a large
21 proportion of animals that tested PCR positive did not show evidence of seroconversion to
22 RVFV as assessed by Elisa is interesting and deserves comments. Firstly, the serology-based
23 detection methods employed have inherent limits to the level of antibody that they can detect.
24 More generally, the scale of the host immune response mounted after an infection is
25 dependant on several host-associated factors including age, health, nutritional status and

1 immuno competence of the host. The pathogen infecting dose and its potential to actively
2 replicate in dendritic cells are also likely to correlate with the magnitude of the invoked
3 immune response, and therefore low-level responses may be expected during inter-epidemic
4 periods, when viral loads are (on the whole) low. During epizootics, occasional animals may
5 prove positive to the pathogen by molecular techniques but fail to show evidence of
6 seroconversion or have delayed seroconversion. In a serosurvey of naturally acquired caprine
7 arthritis encephalitis virus infection in goats, almost 20% of seronegative animals and
8 apparently healthy animals had positive PCR test results and only half of them seroconverted
9 by 8 months later (Rimstad et al., 1993). All these factors are likely operational in the field
10 conditions characteristic of Madagascar and Comoros and may have implications to the
11 epidemiology of RVFV; particularly, whether animals detected PCR positive but failed to
12 seroconvert are contagious and participate to the low level transmission of RVF in the post
13 epidemic period is presently unknown. The new RT-PCR allowed the detection of RVFV in
14 38 samples collected in a global epidemiological network in the Comoros archipelago and
15 Madagascar considered as insular ecosystems (Tortosa et al., 2012). Both serological and
16 molecular findings strongly suggest that the RVF virus is circulating in this area without
17 inducing any clinical signs in infected animals.

18 During IEPs, the epidemiology of RVF remains unclear and involves a possible cryptic
19 enzootic transmission cycle (Linthicum et al., 1985; Bird et al., 2008). Up to now, the
20 detection of the RVF virus in RVF potential species reservoirs such as bats (*Roussetus*
21 *obliviosus*, *Chaerephon pusillus*, *Miniopterus griveaudi*) in Comoros (n=96) and Maki catta
22 (*Lemur catta*) in Mayotte (n=52) was unsuccessful (unpublished data), as well as in
23 Madagascar (Olive et al.; 2013). The question of a possible inside virus propagation via an
24 animal reservoir or a transitory animal host in the Indian Ocean area is still addressed. The

1 use of sensitive detection tools will be a great help in the understanding of the RVF inter-
2 epidemic circulation.

3

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13

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22

1 **Figures captions**

2

3 **Figure 1**

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

11

12 **Figure 2**

13 Comparative efficiency of the Taqman RT-PCR and the new RT-PCR of ten-fold serial
14 dilutions of tissue culture infected supernatant starting from a Smithburn strain stock titrating
15 to 10^{6.3} TCID₅₀/ml. Dilutions detected with (A) the Taqman RT-PCR (Bird et al., 2007) (B)
16 the new RT-PCR .

17

18 **Figure 3**

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

22 Anj: Anjouan, GC: Grande Comore, Mg: Madagascar, Mo: Moheli

23

Table 1
List of viruses tested with the newly developed RT-PCR detection system

Genus	Species	Vector-borne virus	RVF syndrome like associated	Main host
Phlebovirus	Belterra virus	<i>Culicidae</i>	-	Human
	Icoaraci virus	<i>Culicidae</i>	-	Human
	Punta Toro virus	<i>Culicidae</i>	-	Human
	Toscana virus	<i>Phlebotominae</i>	Fever	Human
	Sandfly Naples virus	<i>Phlebotominae</i>	-	Human
	Sandfly Sicilia virus	<i>Phlebotominae</i>	-	Human
Flavivirus	West Nile virus	<i>Culicidae</i>	-	Human/Animal
	Dengue type 1 virus	<i>Culicidae</i>	Fever	Human
	Dengue type 2 virus	<i>Culicidae</i>	Fever	Human
	Dengue type 3 virus	<i>Culicidae</i>	Fever	Human
	Dengue type 4 virus	<i>Culicidae</i>	Fever	Human
	Yellow fever virus	<i>Culicidae</i>	Fever	Human
	Japanese encephalitis virus	<i>Culicidae</i>	Fever	Human
	Apoi virus	NA	-	Human
	Chikungunya virus	<i>Culicidae</i>	Fever	Human
	O'Nyong nyong virus	<i>Culicidae</i>	Fever	Human
Orthobunyavirus	Schmallenberg virus	<i>Ceratopogonidae</i>	Abortion	Animal
Reovirus	Bluetongue virus serotype 2	<i>Ceratopogonidae</i>	Abortion	Animal
Morbillivirus	Peste des petits ruminants virus	NA	Abortion, Diarrhea	Animal
Influenza virus	H5N1 virus A	NA	Fever	Human/Animal

NA: Not Applicable

Table 2

Comparison of two RT-PCR detection systems on a collection of 2,756 serum samples from Madagascar and the Comoros archipelago (May 2010 to August 2011).

Collection area	Number of animals collected during the serosurvey	New RT-PCR, based on the M segment* (% of positive animals)	Taqman RT-PCR (Bird et al., 2007), based on the L segment (% of positive animals)	Incidence levels of seroconversions (%)
Mayotte	104	3/124 (0.29)	0/124 (0)	ND
Union of Comoros	182	20/1063 (10.9)	3/1063 (1.6)	14.4
Madagascar	204	15/1569 (7.35)	2/1569 (0.9)	4.94

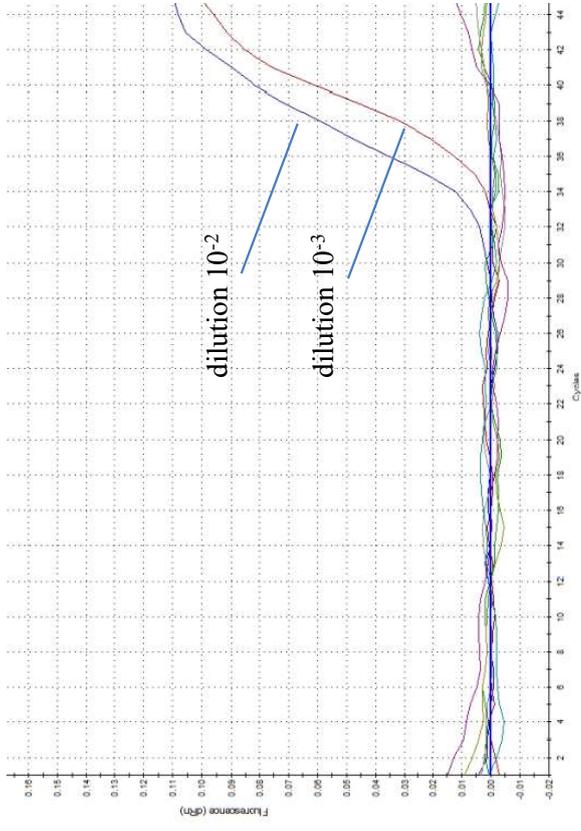
*Each of the positive PCR sample detected with the new RT-PCR on M segment was confirmed by sequence analyses. ND: Not determined

Table 3
Serological status of the 38 RT-PCR positive samples

Sample ID	Month 7 - 11/2010		Month 9 - 01/2011		Month 10 - 02/2011		Month 11 - 03/2011		Month 12 - 04/2011		Month 13 - 05/2011		Month 16 - 08/2011	
	Virology (new RT-PCR)	Serology (ELISA)												
GCCMR0034	+	-	-	+	-	+	-	+	-	+	-	+	-	+
IATV004	+	-	-	+	-	+	-	+	-	+	-	+	-	+
Mg 221 / 2EZM016	-	-	-	+	-	+	-	+	-	+	-	+	-	+
Mg 206 / 1AAR011b	-	-	-	+	-	+	-	+	-	+	-	+	-	+
IAYO015	-	-	+	-	+	-	+	-	+	-	+	-	+	-
Mg 236 / 1ABE013b	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IAJE018	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Mg 222 / 2ETa 023	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Mg 236 / 1ABE012	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Mg 264 / 1AAR009b	-	-	-	-	-	-	+	-	-	-	-	-	-	-
2ERA019b	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Mg 299 / 2FBE001	-	-	-	-	-	-	-	-	+	-	-	-	-	+
Mg 299 / 2FBE003	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Mg 275 / 2FFA030	-	-	-	-	-	-	-	-	+	-	-	-	-	-
GCCMR 0258	-	-	-	-	-	-	-	-	-	-	+	-	-	-
GCBMR 0246	-	-	-	-	-	-	-	-	-	-	+	-	-	-
ABMR 0029	-	-	-	-	-	-	-	-	-	-	+	-	-	-
ABMR 0030	-	-	-	-	-	-	-	-	-	-	+	-	-	-
CVII 28 / GCCMR1017	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 28 / GCCMR1020	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 31 / GCCMR1032	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 40 / GCCSG0539	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 46 / GCCSG0570	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 33 / GCBSG0507	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 39 / GCBSG0536	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 55 / GCBSG0615	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 66 / GCOSG0716	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 15 / ABMR0094	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 20 / ABMR0121	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ABMR 0040	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 3 / MOCMR0071	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVII 3 / MOCMR0072	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2DMA029	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mg314 / 2DNJ023	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SG 134	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MJ 15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abortion	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Samples detected by the Taqman RT-PCR (Bird et al., 2007)

Figure(s)
A.



B.

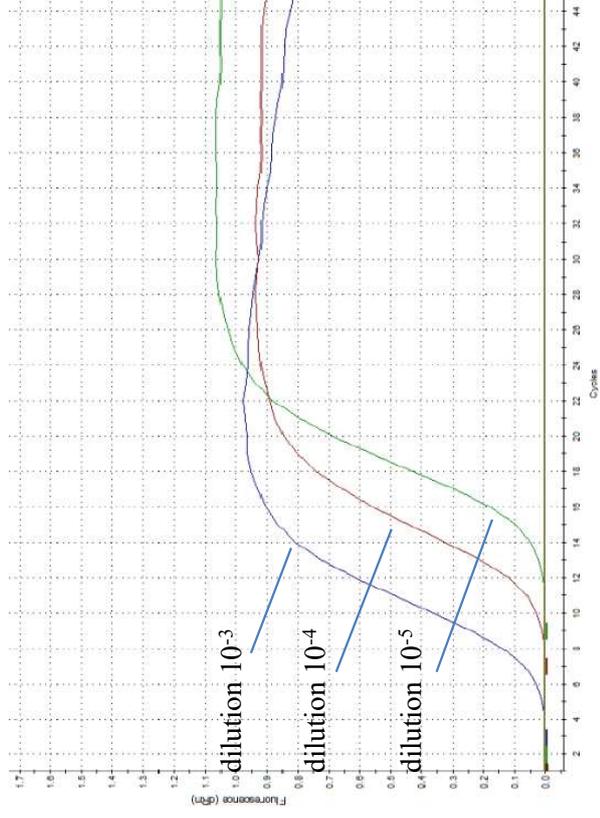


Figure 2

Figure(s)

Consensus	Genbank accession N°	2,660	2,680	2,700	2,720	2,740	2,760
Burkina faso 1983	DQ380187	C					
Central African Republic 1969, Zinga	DQ380217						
Central African Republic 1974	DQ380212		C				
Central African Republic 1985	DQ380219						
Clone 13	DQ380213		C				
Egypt 1977	NC 014396						
Egypt 1977, ZH548	DQ380206						
Egypt 1978	DQ380204						
Egypt 1978	DQ380207						
Egypt 1979	DQ380205						
Guinea 1981	DQ380215						
Guinea 1984	DQ380216						
Kenya 1957, Rintoul	DQ380192				C		
Kenya 1983	DQ380198				U		U
Kenya 2006	JF326192						U
Kenya 2006	JF326193						U
Kenya 2006	HM586972						U
Kenya 2007	HM586966						U
Kenya 2007	EU574050						U
Kenya 2007	EU574053						U
Kenya 2007	EU574054						U
Kenya 2007	EU574056						U
Madagascar 1951	JF311382						U
Madagascar 1979	DQ380210						U
Madagascar 1991	JF311381		U				U
Madagascar 2008	HQ009512						U
Madagascar 2008	JF311377						U
Madagascar 2008	JF311378						U
Madagascar 2008	JF311384						U
Madagascar 2208	JF311385						U
Mauritania 1987	DQ380184	C		U			
Mauritania 1987	DQ380185	C		U			
Mauritania 2007	DQ380183	C		U			
Mayotte 2008	HE687303						U
Mayotte 2008	HE687306						U
MP-12	DQ380208						U
Saudi 2000	DQ380197						U
Smithburn	DQ380193		C			C	
South Africa 1951	DQ380195			U			
South Africa 1974	JF784387						
South Africa 1975	DQ380189				C		C
South Africa 2009	JQ068143				C		C
Sudan 2010	JQ820487						U
Sudan 2010	JQ820489		C				U
Sudan 2012	JQ820488		C				U
Tanzania 2007	HM586970						U
Tanzania 2007	JF326194						U
Tanzania 2007 3	JF326195						U
Uganda 1944, Entebbe	DQ380191						C
Zimbabwe 1970	DQ380188					C	C
Zimbabwe 1974	DQ380209						
Zimbabwe 1978	DQ380220						
Anj ABMR 029				G			C
Anj ABMR 030				C		A	A
Anj ABMR 040				C		A	A
Anj CVII14			G	G	T		CC
Anj CVII15			G	G	T		CC
Anj CVII20							C
GC CVII28				A			C
GC CVII31							C
GC CVII33							C
GC CVII39							C
GC CVII39							C
GC CVII40							C
GC CVII46				G			CC
GC CVII55							C
GC CVII66							C
Mayotte 2010 Av							C
Mayotte 2010 MJ15							C
Mayotte 2010 Sg134							C
Mg 1 AJE018							C
Mg 1 ATV004							C
Mg 206							C
Mg 222							C
Mg 236							C
Mg 264							C
Mg 275							C
Mg 299							C
Mg 2DMa 029 bis							C
Mg 314							C
Mg 221							C
Mn CVII3				G			CC

Fig.3.

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