Supplementary data

Suppl. S1: List of primers used for the completion of genome and screening

Description	Primer name	Primer sequence (5'→3')
Screening primers for human and bat RVAs	RVA-VP6_40F	GCK AGR GAC AAR ATT GTY GAA GG
	RVA-VP6_1083R	GWC CAA TTC ATR CCT GGT GGA
Primers for completing segments of RVA/Bat-wt/CMR/BatLy03/2014/G25P[43]	RVA59_VP1_301-322R	TTA GCT AAT TTA CCC TCA ACG G
	RVA59_VP1_328-349F	AAG CTA ACG TCA GAA TTA TTC GC
	RVA59_VP1_737-758R	GCT ACT AAG ATT GAC ATT GGT G
	RVA59_VP1_2991-3013F	GTC AAT AAA CTA TGG CTG TTA CC
	RVA59_VP1_3048-3072F	ATT AAT TCG TAT ACC CTT CAA AGG T
	RVA59_VP3_76-100F	CAA TCT TAT GCT GAT ACT CAA ACG
	RVA59_VP3_787-809R	TGT CAA ATT GTG AAA TAC GTC GC
	RVA59_VP3_1795-1818F	CGT TAT CTG GTT ACA TAT TTA GAG
	RVA59_VP4_2063-2084F	GAA CAG ATG GTA GAT TTT TCG C
	RVA59_VP4_171-195R	TAC AGT TGT TGA ATC GTT AAT TTC C
	RVA59_NSP1_1433-1453F	AAA TCA CTC TTA TTC CGG TGG
	RVA59_NSP5_389-411F	ATC AAT TAC TAC GGA TCA TGC TG
Primers for completing segments of RVA/Bat-wt/CMR/BatLi10/2014/G30P[42]	RVA46_VP4_118-137_F	TTA CGC ACC AGT GAA TTG GG
	RVA46_VP4_839-860_R	ACC ACT TAT ATC CTA AAC CAC C
	RVA46_VP7_172-191_F	TCA ATG GAC GTT GTG TTG GC
	RVA46_VP7_803-823_R	GAT TTG TTG TTG GAT CTG ACG
Primers for the amplification of 5'and 3' ends of VP7 and VP4 segments	RVA63_VP7-5'-227_R	TTA CTAGAC TCA TGT CCA TCG
	RVA63_VP7-3'-839_F	AGT ATT ACA AGT TGG AGG TGC
	RVA894_VP7-5'-202_R	TGT AAC CGG TAT GTT CAA TCC
	RVA894_VP7-3'-788_F	GTA AGA AAA TCG GAC CTA GAG
	RVA59_VP7-5'-213_R	TCC ATT GAT CCA GTA ATT GGC
	RVA59_VP7-3'-843_F	TAT AAC AGC AGA TCC AAC GAC
	RVA63_VP4-5'-230_R	ATA CCC AAT TAT CGA CTG CTG
	RVA63_VP4-3'-2143_F	CTT GTA ACT GAT TCA CCA GTC
	RVA59_VP4-5'-214_R	AAA TTC TGT TGG CTG ATA GGG
	RVA59_VP4-3'-2100_F	AGA TTT TTC GCA TAT AAA GTG AGC
	RVA894_VP4-5'-158_R	TAC TGT TGA GTC ATT TAC TTC CC
	RVA894_VP4-3'-2072_F	GAA CAG ATG GTA GAT TTT TCG C

Suppl. S2: Nucleotide percentage similarity between primers and corresponding sequences of Cameroonian bat RVA strains. -: 5’ or 3’-end amplification failed.

Name of primer	BatLy03 G25P[43]	BatLi08 G31P[42]	BatLi09 G30P[42]	BatLy17 G30P[xx]	BatLi10 G30P[42]	Reference (PMCID)
Outer capsid glycoprotein VP7 forward primers						
Beg9	96.4	57.1	-	85.7	-	269590
sBeg9	100	57.1	-	90.5	-	269590
9Con1-L	100	100	-	100	-	263808
Outer capsid glycoprotein VP7 reverse primer						
EndA	78.2	91.3	95.7	-	-	10364621
VP7-Rdeg	95.0	95.0	100	-	-	114861
End9	85.1	66.7	59.3	-	-	269590
RVG9	89.5	63.2	63.2	-	-	269590
Outer capsid protein VP4 forward primers						
VP4-1-17F	88.8	88.8	77.8	94.4	-	3295124
Con3	72.7	68.2	72.7	63.6	72.7	265294
Outer capsid protein VP4 reverse primer						
Con2	85.7	81.0	81.0	76.2	81.0	265294

Suppl. S3: Detail explanations of the comparisons between screening primer pairs and the novel bat VP4 and VP7 genotypes

VP7 forward primers Beg9, sBeg9 and 9Con1-L showed a (near) perfect match with BatLy03-G25, whereas strain BatLi08-G31 and BatLy17-G30 (first 6 nt are missing for this strain), showed up to 10 and 4 nucleotide mismatches at the 3’end of the primers, respectively. VP7 forward primer 9con1-L showed a perfect match with all the genotypes (G25, G30 and G31). Considering the VP7 reverse primers EndA, VP7-Rdeg, End9 and RVG9, BatLy03-G25 did not show a perfect match as there were 4, 1, 4 and 2 mutations, respectively. The mismatches with EndA, VP7-Rdeg and RVG9 were near the middle or at the 5’end of the primer, whereas 2 of those of End9 were close to the 3’end. Comparing the same VP7 reverse primers with strain BatLi08-G31 and BatLi09-G30 also showed mismatches. For EndA and VP7-Rdeg maximum 2 mismatches are located in the middle or near the 5’-end, whereas for End9 and RVG, there

were multiple mismatches of which 2 and 7 mismatches, respectively were right at the 3'-end. For VP4 forward primer VP4-1-17F, BatLy03-P[43], BatLi08-P[42], BatLy17-P[xx] and BatLi09-P[42] showed 2, 2, 1 and 2, mismatches, respectively, with at least a mutation at the first position from the 3'end for all of them. For con3, there were 6, 7, 6 and 8 mismatches with BatLy03-P[43], BatLi08-P[42), BatLi09-P[42] and BatLy17-P[xx], respectively. Considering the VP4 reverse primer con2, strains BatLy03-P[43], BatLi08-P[42], BatLi09-P[42], BatLy17-P[xx] and BatLi10-P[42] showed 3-5 mismatches including one at the second position from the 3'-end.

Suppl. S4: Phylogenetic trees of nucleotide sequences of the RVA VP6 sequence. HRVA: Human RVA VP6 sequence from patients exposed to bats; open triangle: Cameroonian bat RVA strains; Other bat RVA: KE4582, MSLH14 and MYAS33. Bootstrap values (1000 replicates) above 70% are shown. Scale represent number of nucleotide substations per site.