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No Evidence of Gouléako and Herbert Virus Infections in Pigs, Côte d'Ivoire and Ghana

Technical Appendix

Additional Methods and Results

Growth Kinetics

Porcine kidney (PK)-15 cells, human embryonic kidney (HEK)-293 cells, and human hepatocellular carcinoma (HuH)-7 cells were infected with Gouléako virus (GOLV), Herbert virus (HEBV), and vesicular stomatitis virus (VSV) as a positive control at multiplicities of infection (MOI) of 1, as described (1,2). Cell culture supernatants were analyzed for viral genome copy numbers at 0, 3, and 6 days postinfection by real-time reverse transcription PCR (1,2).

Amplification of GOLV Glycoprotein Precursor Gene Sequences

RNA was extracted from infected C6/36 cells by using the Viral RNA Kit (QIAGEN, Hilden, Germany) and cDNA synthesis was performed by using SuperScript III (Thermo Fisher Scientific, Lithuania) Glycoprotein precursor gene fragments were amplified by using primers based on strain GOLV/A5/CI/2005 and Platinum Taq polymerase, according to the manufacturer's instructions (Thermo Fisher Scientific, Lithuania). PCR products were analyzed by agarose gel electrophoresis and sequenced by Seqlab (Göttingen, Germany). Sequences were deposited in the GenBank database (National Center for Biotechnology Information, Bethesda, MD, USA) under accession number KT387771–KT387796.

Phylogenetic Analyses

GOLV glycoprotein precursor gene and HEBV RdRp sequences were aligned by using the multiple sequence alignment program MAFFT (http://wiki.hpc.ufl.edu/doc/PhyML); maximum likelihood analyses were inferred by using PhyML (https://code.google.com/p/phyml/) with the HKY85 substitution matrix and 1,000 bootstrap replicates in Geneiuos (Biomatters, Aukland, New Zealand; http://www.geneious.com/).

PCR Screening of Swine Serum Samples

Ethical review and clearances of animal handling procedure were obtained from the Ghana Forestry Commission of the Ministry of Food and Agriculture. RNA was extracted from 15μ L of porcine serum samples mixed with 55μ L Dulbecco's Phosphate-Buffered Saline by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Random cDNA synthesis was performed by using SuperScript III (Thermo Fisher Scientific, Lithuania). Viral genome copies were measured by real-time reverse–transcription PCR, as described previously (*1*,*2*). The Technical Appendix Table shows samples tested and results.

Recombinant Nucleocapsid Immunofluorescence Assay (IFA)

Porcine serum samples were screened for presence of antibodies against the GOLV and HEBV viruses in 1:20 dilutions by rIFA as described (*3*). C-terminally FLAG-tagged full nucleocapsid genes of GOLV or HEBV were amplified from cDNA by using the primers GOLV-N-XbaI-F (5'-

GCTCTAGAGCCACCATGGCAACAGTTACTCAGAATGACATTCAG), GOLV-N-FLAG-C-XbaI-R (5'-GCTCTAGATCACTTGTCATCGTCGTCCTTGTAGTCACCAGCTTCCATCAGTTTTCC GGCCGC), HEBV-N-*Bam*HI-F (5'-CGGGATCCGCCACCATGGCTACCAATTTTGAATTCAATGATAAC), and HEBV-N-

FLAG-C-SphI-R (5'-ACATGCATGCTCACTTGTCATCGTCGTCCTTGTAGTCACCAGCTTGAGGCCATATT TTGTTGATCAGTG). The amplified genes were then cloned into a pCG1 eukaryotic expression vector.

Plasmids were sequence confirmed. Transfected cells were used in indirect immunofluorescence assays with goat anti-swine IgG-Alexa Fluor 488 conjugate (Sigma, St. Louis, USA) in 1:200 dilution for detection of bound swine serum antibody. A rabbit anti-FLAG antibody and goat anti-rabbit fluorescein-labeled conjugate in 1:200 dilution (Dianova, Hamburg, Germany) were used to confirm expression of viral proteins. A cterminal flag tag will be expressed only when the upstream viral protein ORF is intact. Cell nuclei were stained with ProLong Gold Antifade Mountant (Thermo Fisher Scientific, Lithuania) with DAPI (4', 6-diamidino-2-phynylindole).

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Technical Appendix Table. Porcine serum samples tested for infection with GOLV and HEBV by using real-time reverse transcription PCR and rIFA, in Côte d'Ivoire and Ghana, 2008–2011*

		Age,	Age, rifa		FA	Virai RNA		
Sample ID	Sex	mo†	Origin	Year	GOLV	HEBV	GOLV	HEBV
CI-S 01	Male	12	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 02	Female	12	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 03	Female	12	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 04	Male	11	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 05	Female	9	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 06	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 07	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 08	Male	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 09	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 10	Female	6	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 11	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 12	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 13	Female	12	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 14	Male	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 15	Female	8	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 16	Female	4	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 17	Female	4	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 18	Female	2	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 19	Male	2	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 20	Female	4	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 21	Male	4	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 22	Female	2	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 23	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 24	Female	2	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 25	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 26	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 27	Female	3	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
CI-S 28	Female	5	Gouléako, Cl	2008	Neg	Neg	Neg	Neg
GH-S 01	Female	24	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 02	Female	6	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 04	Male	6	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 05	Female	36	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 06	Male	6	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 07	Male	6	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 08	Female	8	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 09	Female	8	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 10	Female	7	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 11	Female	7	Amanfrom, GH	2011	Neg	Neg	Neg	Neg
GH-S 12	Female	1.5	Sokoban New Town, GH	2011	Neg	Neg	Neg	Neg
GH-S 13	Male	6	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 14	Male	8	Dompoase, GH	2011	Neg	Neg	Neg	Neg

		Age,			rIFA		Viral RNA	
Sample ID	Sex	mot	Origin	Year	GOLV	HEBV	GOLV	HEBV
GH-S 15	Male	6	Dompose CH	2011	Neg	Neg	Neg	Neg
	Fomolo	1 5	Sekehan New Town CH	2011	Neg	Neg	Neg	Neg
GH-5 16	Female	1.5	Sokoban New Town, GH	2011	Neg	Neg	iveg	Neg
GH-S 17	Female	6	Sokoban New Town, GH	2011	Neg	Neg	Neg	Neg
GH-S 18	Male	1.5	Sokoban New Town, GH	2011	Neg	Neg	Neg	Neg
GH-S 19	Male	6	Sokoban New Town, GH	2011	Neg	Neg	Neg	Neg
GH-S 20	Male	6	Sokoban New Town, GH	2011	Nea	Nea	Nea	Nea
GH-S 21	Female	8	Dompoase GH	2011	Neg	Neg	Neg	Neg
	Molo	6	Domposoo, CH	2011	Nog	Nog	Nog	Nog
GH-5 22	iviale	0	Dompoase, GH	2011	Neg	iveg	iveg	iveg
GH-S 23	Male	6	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 24	Male	6	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 25	Female	6	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 26	Male	8	Dompoase, GH	2011	Nea	Nea	Nea	Nea
GH-S 27	Female	õ	Dompoase GH	2011	Neg	Neg	Neg	Neg
	Fomolo	0	Dompoase, CH	2011	Neg	Neg	Neg	Neg
GH-5 20	remale	<u> </u>	Dompoase, GH	2011	Neg	ineg	Neg	Neg
GH-S 31	Male	5	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 32	Male	5	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 33	Female	5	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 34	Male	5	Akropong, GH	2011	Nea	Nea	Nea	Nea
GH-S 35	Male	5	Akropong GH	2011	Neg	Neg	Neg	Neg
	Male	5	Akropong, CH	2011	Nog	Nog	Nog	Nog
011-0-07		5	Akiopolig, GH	2011	Ney	Neg	Neg	Neg
GH-S 37	Female	5	Akropong, GH	2011	ineg	ineg	ineg	Neg
GH-S 38	Female	7	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 39	Female	7	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 40	Male	6	Akropona, GH	2011	Nea	Nea	Nea	Nea
GH-S 41	Male	6	Akronong 2 GH	2011	Neg	Neg	Neg	Neg
	Malo	6	Akropong 2, CH	2011	Nog	Nog	Nog	Nog
GH-3 42	Iviale	0	Aktopolig 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 43	Male	6	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 44	Female	6	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 45	Female	6	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 46	Male	5	Akropona, GH	2011	Nea	Nea	Nea	Nea
GH-S 47	Female	5	Akropong GH	2011	Neg	Neg	Neg	Neg
	Mala	5	Akropong, CH	2011	Nog	Nog	Nog	Nog
011-0 40		5	Akiopolig, GH	2011	Neg	Neg	Neg	Neg
GH-5 49	Female	5	Akropong, GH	2011	ineg	ineg	Neg	Neg
GH-S 50	Female	6	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 51	Female	6	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 52	Male	6	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 53	Male	7	Akropong 2 GH	2011	Neg	Neg	Neg	Neg
CH-S 54	Male	7	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
	Fomolo	7	Akropong 2, CH	2011	Neg	Neg	Neg	Neg
GH-5 55	Female	7		2011	Neg	Neg	Neg	Neg
GH-S 56	Female	1	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 57	Male	7	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 58	Male	5	Essienimpong, GH	2011	Neg	Neg	Neg	Neg
GH-S 59	Female	6	Essienimpong, GH	2011	Nea	Nea	Nea	Nea
GH-S 60	Male	5	Essienimpong GH	2011	Neg	Neg	Neg	Neg
	Male	5		2011	Nog	Nog	Nog	Nog
GH-5 01	iviale	D A	Essienimpong, GH	2011	Neg	ineg	Neg	Neg
GH-5 62	Male	4	Akropong 2, GH	2011	ineg	ineg	Neg	Neg
GH-S 63	Female	4	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 64	Male	4	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 65	Female	4	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 66	Female	4	Akropong 2, GH	2011	Nea	Nea	Nea	Nea
GH-S 67	Male	5	Essienimpong GH	2011	Neg	Neg	Neg	Neg
	Eomolo	5	Essionimpong, CH	2011	Nog	Nog	Nog	Nog
011-3 00	Female	5	Essienimpolig, GH	2011	Neg	Neg	Neg	Neg
GH-5 69	Female	5	Essienimpong, GH	2011	ineg	ineg	iveg	Neg
GH-S 70	Female	5	Essienimpong, GH	2011	Neg	Neg	Neg	Neg
GH-S 71	Female	6	Essienimpong, GH	2011	Neg	Neg	Neg	Neg
GH-S 72	Female	6	Essienimpong, GH	2011	Neg	Neg	Neg	Neg
GH-S 73	Female	8	Onwe, GH	2011	Nea	Nea	Nea	Nea
GH-S 74	Female	8	Onwe GH	2011	Neg	Neg	Neg	Neg
	Fomalo	0		2011	Nog	Nog	Nog	Nog
	Mala	0		2011	Neg	Neg	Neg	Neg
GU-9 /0	iviale	ð	Onwe, GH	2011	ineg	ineg	iveg	iveg
GH-S //	_iviale	8	Onwe, GH	2011	ineg	ineg	ineg	ineg
GH-S 78	Female	5	Onwe, GH	2011	Neg	Neg	Neg	Neg
GH-S 79	Male	8	Onwe, GH	2011	Neg	Neg	Neg	Neg
GH-S 80	Female	8	Onwe. GH	2011	Nea	Nea	Nea	Nea
GH-S 81	Female	5	Onwe GH	2011	Neg	Neg	Neg	Neg
CH-C 82	Fomalo	5		2011	Nog	Nog	Nog	Neg
	Female	5		2011	Neg	Neg	Neg	Neg
GH-2 23	remale	<u>/</u>	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg
GH-S 84	⊢emale	1	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg
GH-S 85	Female	7	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg
GH-S 86	Female	7	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg
GH-S 87	Male	7	Ejisu Krapa, GH	2011	Nea	Nea	Nea	Nea
GH-S 89	Female	5	Fijsu Krana, GH	2011	Neg	Neg	Neg	Neg
GH-6 00	Female	5	Ejisu Krana GH	2011	Neg	Neg	Neg	Neg
	i cinale	5	∟μου Μαρά, ΟΠ	2011	iveg	ivey	ivey	incy

		Age,			rIFA		Viral RNA		
Sample ID	Sex	mo†	Origin	Year	GOLV	HEBV	GOLV	HEBV	
GH-S 91	Male	4	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg	
GH-S 92	Female	5	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg	
GH-S 93	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 94	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 95	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 96	Male	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 97	Male	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 98	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 99	Male	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 100	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 101	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 102	Male	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 103	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 104	Female	5	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 105	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 106	Female	4	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 107	Male	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
GH-S 108	Female	12	Abattoir, GH	2011	Neg	Neg	Neg	Neg	
*CI, Côte d'Ivo	ire; GH, Ghan	a; GOLV, Gou	uléako virus; HEBV, Herbert vi	rus; Neg, Neg	gative results.	-	-	-	
†Age is age of pig from which serum sample was collected.									

Contraction of the second seco

GOLV-N

HEBV-N

Technical Appendix Figure 1. Immunofluorescence patterns for antibodies against Gouléako virus (GOLV) and Herbert virus (HEBV) in serum samples from swine, Côte d'Ivoire (CI), 2008, and Ghana, 2011. Figure shows representative results from 1 pig (labeled CI-S 09) from which serum was tested against overexpressed recombinant nucleocapsid protein of A) GOLV and B) HEBV in VeroB4 cells. Anti-FLAG IgG antibodies were used to control for overexpression of C) GOLV-nucleocapsid (N) and D) HEBV-N. Scale bar indicates 20 μm. All photographs were taken at equivalent exposure settings.

A Nucleocapsid protein	1 MATVTQNDIQEAQAYI	ASVSLLALSDG	30 DLV LS VEK LE Y	⁴⁰ OGFDPYAFLS	⁵⁰ Y <mark>L</mark> FAVAKKA	GIGEAEHKK	70	⁸⁰ MRGGKAKKIA <mark>B</mark>	90 100 KSTPETKRWLESMI	<u>E QK</u>
1. GOLV_strain A5/CI/2004_HQ541736 2. GOLV_strain F23/CI/2004_EF423169 3. GOLV_strain CP13-1/2013_KJ830623 4. GOLV_strain CP13-2/2013_KJ830624	MATVTQNDIQEAQAYI MATVTQNDIQEAQAYI MATVTQNDIQEAQAYI MATVTQNDIQEAQAYI MATVTQNDIQEAQAYI	ASVSLLALSDG ASVSLLALSDG ASVSLLALSDG ASVSLLALSDG	DLVLSVEKLEY DLVLSVEKLEY DLVLSVEKLEY DLVLSVEKLEY	QG FDPYAFLS QG FDPYAFLS QG FDPYAFLS QG FDPYAFLS 140	YLFAVAKKA YLFAVAKKA YLFAVAKKA YLFAVAKKA	GIGEAEHKK GIGEAEHKK GIGEAEHKK GIGEAEHTK	LQTLAVLGT LQTLAVLGT LQTLAVLGT LQTLAVLGT	MRGGKAKKIAE MRGGKAKKIAE MRGGKAKKIAE	KSTPETKRWLESMI KSTPETKRWLESMI KSTPETKRWLESMI KSTPETKRWLESMI	I QK I QK I QK I QK
Consensus Identity	MIQKYSITSGRPTGSI	K DV TLLR IAA CH	AA <mark>PIAIGISTG</mark>	LAVKTTINPR	SXHENYXPY	MCISTFGSL	IPVVGTGLSS	DDVR LISDAFT	YHQRLFDRVINPR.	APN
1. GOLV_strain A5/CI/2004_HQ541736 2. GOLV_strain F23/CI/2004_EF423169 3. GOLV_strain CP13-1/2013_KJ830623 4. GOLV_strain CP13-2/2013_KJ830624	MIQKYSITSGRPTGSI MIQKYSITSGRPTGSI MIQKYSITSGRPTGSI MIQKYSITSGRPTGSI	K DV TLLRIAACH K DV TLLRIAACH K DV TLLRIAACH K DV TLLRIAACH	AAPIAIGISTG AAPIAIGISTG AAPIAIGISTG AAPIAIGISTG	LAVKTTINPR LAVKTTINPR LAVKTTINPR LAVKTTINPR	SIHENYPPY SIHENYPPY SMHENYAPY SMHENYAPY	MCISTFGSL MCISTFGSL MCISTFGSL MC <mark>E</mark> STFGSL	I PVVGTGLSS I PVVGTGLSS I PVVGTGLSS I PVVGTGLSS	DDVRLISDAFT DDV DDVRLISDAFT DDVRLISDAFT	YHQMLFDRVINPR. YHQRLFDRVINPR. YHQRLFDRVINPR.	A PN A PN A PN
Consensus Identity	200 210 APNSKETLKSYVDIQ	220 YMSGLYEPEMRL	QVCMK LGLI TG	240 ARGTYTINAC	250 VK PALQHAA	CELLE				
1. GOLV_strain A5/Cl/2004_HQ541736 2. GOLV_strain F23/Cl/2004_EF423169 3. GOLV_strain CP13-1/2013_KJ830623 4. GOLV_strain CP13-2/2013_KJ830624	<pre>{A PNSK ETLKS YV DIQ {A PNSK ETLKS YV DIQ {A PNSK ETLKS YV DIQ</pre>	YMSGLYEPEMRL YMSGLYEPEMRL YMSGLYEPEMRL	QVCMKLGLITG QVCMKLGLITG QVCMKLGLITG	ARGTYTINAG ARGTYTINAG ARGTYTINAG	VK PALQHAA VK PALQHAA VK PALQHAA	GKLME GELLE GELLE				
B Glycoprotein precursor p	protein	Gn protein (47	'4 aa)				Gc proteir	n (489 aa)		
	1 100	200	300	400	500 soo	600	700	800	900	968 968
Consensus Identity										
C Glycoprotein precursor	protein									
Consensus Identity	CKAPCSKKSLHISG	LLESMGYIALS	NASHIQLS P	EESHLGWGN	WLKGVWFS	MGWFNVLW		FVLSLMHKI	KVGSKVKIW	
 COLV_strain_A05/C//2004 COLV_strain_A05/C//2004 COLV_M_F26M-C-2004 COLV_Strain_A28/C//2004 COLV_strain_A28/C//2004 COLV_strain_A30/C//2004 COLV_strain_A30/C//2004 COLV_strain_A30/C//2004 COLV_strain_A50/C//2004 COLV_strain_A50/C//2004 COLV_strain_A50/C//2004 COLV_strain_A50/C//2004 COLV_strain_C40/C/2004 COLV_strain_C50/C//2004 COLV_strain_C65/C//2004 COLV_strain_C65/C//2004 COLV_strain_C65/C//2004 COLV_strain_C65/C//2004 COLV_strain_C65/C//2004 COLV_strain_D50/C//2004 COLV_strain_D50/C//2004 COLV_strain_F25/C//2004 COLV_strain_F26/C//2004 COLV_strain_F26/C//2004 COLV_strain_F26/C//2004 COLV_strain_F30/C//2004 COLV_strain_F30/C//2004 COLV_strain_F30/C//2004 COLV_strain_F26/C//2004 COLV_strain_F26/C//2004 COLV_strain_F26/C//2004 COLV_strain_F30/C//2004 COLV	CKA P C SKKS L H I S G CKA P C SKKS L H S G CKA P C	LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL LLESMGYIAL	N NA S HI OLS P NAS HI OLS P	ESHLGWGN ESHLGWGN	M L K Q V W ES M L K	M G W FN V L W M G W FN V L W M G W FN V L W M G W FN V L W W M G W F	IS LG LI LV IS LG LI LV	FVLSLMHKI FVLSLMHKI		

Technical Appendix Figure 2. Alignment of Gouléako virus (GOLV) strains from mosquitoes originating from Côte d'Ivoire and Ghana and swine sampled in Korea. A) Alignment of the GOLV nucleocapsid proteins; B) alignment of complete GOLV glycoprotein precursor protein from mosquito and of protein fragments identified in swine. Schematic overview of encoded proteins is shown in boxes. Transmembrane domains are marked in yellow. Protein domains located outside virions are shown in dark blue, and those located inside virions are in light blue. C) Alignment of Gc proteins originating from mosquitoes and swine. A red line (at consensus identity 29 and 30) marks sequences from swine published by Chung et al. in 2014 (*4*).



Technical Appendix Figure 3. Alignment of Herbert virus (HEBV) strains from mosquitoes and pigs. A) Overview of location of amplified RNA-dependent RNA polymerase (RdRp) protein sequences available from mosquitoes and swine. Sequences detected in swine in South Korea are indicated by a red line. B) Alignment of RdRp sequences from mosquitoes and swine. Amino acid changes are colored. Sequences detected in swine in South Korea are indicated by a red line. C) Alignment of HEBV protein sequences of the third conserved region of the RdRp identified in mosquitoes from Côte d'Ivoire and Ghana.