

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

Sandra Junglen, Marco Marklewitz,  
Florian Zirkel, Robert Wollny, Benjamin Meyer,  
Hanna Heidemann, Sonja Metzger,  
Augustina Annan, Dickson Dei,  
Fabian H. Leendertz, Samuel Oppong,  
Christian Drosten

A recent report suggested that 2 novel bunyaviruses discovered in insects in Côte d'Ivoire caused lethal disease in swine in South Korea. We conducted cell culture studies and tested serum from pigs exposed to mosquitoes in Côte d'Ivoire and Ghana and found no evidence for infection in pigs.

Orthobunyaviruses and phleboviruses are transmitted to animals and humans by blood-feeding arthropods such as mosquitoes, sandflies, and ticks (1,2). Infection can cause systemic disease, including encephalitis or hemorrhagic fevers. Members of both genera of viruses encode a nonstructural (NS) protein that suppresses the antiviral interferon response of the vertebrate host (3,4). We recently discovered 2 novel prototypic bunyaviruses in mosquitoes in Côte d'Ivoire (5,6). Named Gouléako virus (GOLV) and Herbert virus (HEBV), the viruses tentatively define 2 novel bunyavirus-family genera that are in a sister relationship to the genera *Phlebovirus* and *Orthobunyavirus*, respectively. Neither virus encodes NS proteins, nor do the viruses infect vertebrate cells or cause disease in mice that have been intracerebrally inoculated with the viruses (5–7). Replication of both viruses is blocked at temperatures above 31°C, suggesting that the viruses are unlikely to infect mammals (8).

Chung et al. recently reported that, in 2013, GOLV and HEBV caused prevalent and lethal infections in swine in South Korea (9). In that study, >500 pigs from 40 farms were tested for both viruses, and viral RNA was detected in up to 79% of diseased and 55% of healthy pigs. Dead pigs carried virus in their lungs and intestines. GOLV was isolated from swine serum in porcine kidney 15 cells. These

results suggest the discovery of disease caused by these 2 novel viruses in a major livestock species. Because of the implications of this finding, we attempted verification.

## The Study

We first extended our recent cell culture studies to include porcine kidney 15 and human embryonic kidney 293 cells, which were the type of cells used by Chung et al. (9). Human hepatocellular 7 carcinoma cells were also included because they are highly susceptible to virus infection, as are Vero cells and several other cell lines we used in earlier studies (5,6). Infections with GOLV and HEBV were performed at multiplicities of infection of 1 in doublets in all cell lines. Vesicular stomatitis virus was used as a positive control at multiplicity of infection 1. Cell culture supernatants were analyzed for viral RNA after 0, 3, and 6 days by real-time reverse transcription PCR (RT-PCR) (5,6). No replication of GOLV and HEBV was detected, whereas vesicular stomatitis virus replicated to high concentrations (Figure 1). Three blind passages on fresh cells failed to yield virus.

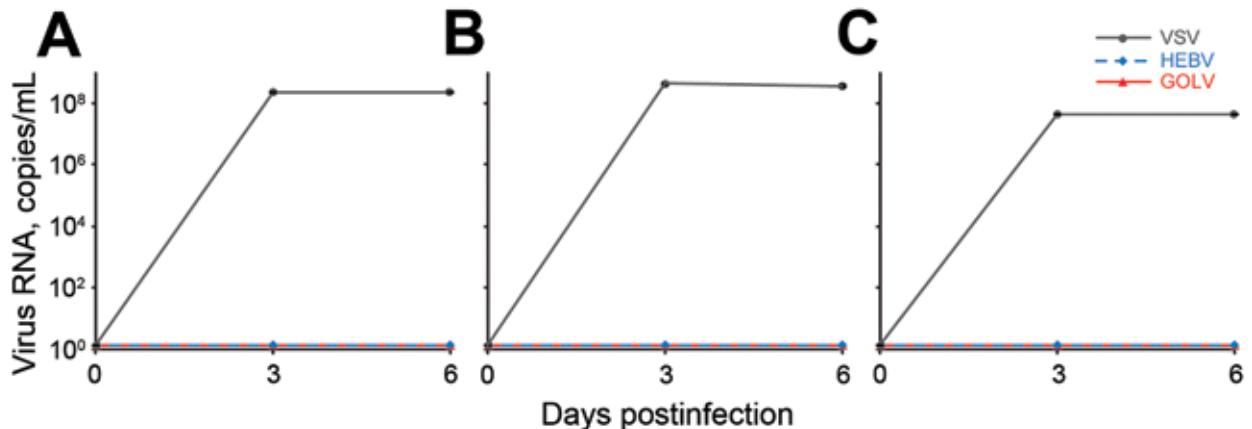
Because cell culture experiments may not show the full host range of a specific virus, we tested serum samples collected in 2008 from *Sus scrofa domestica* pigs in Gouléako, the rural village where GOLV and HEBV were first isolated from mosquitoes in Côte d'Ivoire (5,6). The 28 tested samples represented nearly all the pigs kept in Gouléako at that time, all of which were constantly exposed to mosquitoes. We also tested 108 serum samples collected in 2011 from mosquito-exposed swine in Kumasi, Ghana, where mosquitoes were found to be infected with HEBV (6) and GOLV (S. Junglen, unpub. data).

All samples were tested for virus by real-time RT-PCR (5,6) and tested for antibodies against GOLV and HEBV nucleocapsid proteins by recombinant immunofluorescence assay (10). All samples were negative for the viruses (online Technical Appendix Table, <http://wwwnc.cdc.gov/EID/article/21/12/14-1840-Techapp.pdf>). Online Technical Appendix Figure 1 shows antigen controls and results from 1 representative swine serum sample.

To compare the viruses found in pigs in South Korea with viruses found in mosquitoes in Africa, we replicated methods used by Chung et al. (9) and amplified a region of the GOLV glycoprotein precursor gene from 27 GOLV strains in mosquitoes (online Technical Appendix). Nucleotide sequence distance among mosquito strains was as high as 9.0%. The viruses found in the pigs fell within the genetic diversity of viral strains of GOLV and HEBV and

Author affiliations: University of Bonn Medical Center, Bonn, Germany (S. Junglen, M. Marklewitz, F. Zirkel, R. Wollny, B. Meyer, H. Heidemann, C. Drosten); Robert Koch Institute, Berlin, Germany (S. Metzger, F.H. Leendertz); Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana (A. Annan); Ghana Veterinary Services, Kumasi (D. Dei); Kwame Nkrumah University of Science and Technology, Kumasi (S. Oppong)

DOI: <http://dx.doi.org/10.3201/eid2112.141840>



**Figure 1.** Infection of cells with vesicular stomatitis virus (VSV), Herbert virus (HEBV), and Gouléako virus (GOLV). A) Porcine kidney 15 cells; B) human embryonic kidney cells; C) human hepatocellular 7 cells. Cells were infected at a multiplicity of infection of 1. The number of viral genome copies in cell culture supernatants were measured at 0, 3, and 6 days postinfection by real-time reverse transcription PCR.

did not constitute phylogenetic outliers (Figure 2, panel A). The analyzed fragment had 6 aa exchanges, but they were insufficient for drawing conclusions about protein function because the fragment did not include domains putatively relevant for receptor binding (online Technical Appendix Figure 2).

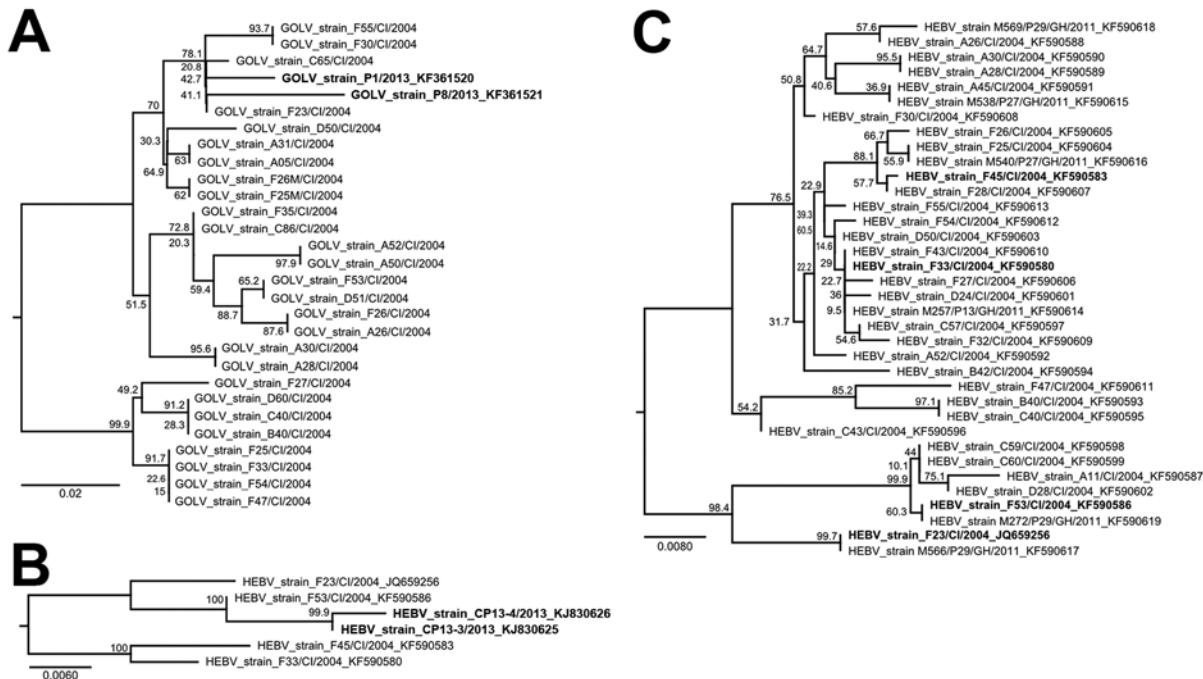
Small RT-PCR fragments from the RNA-dependent RNA polymerase (RdRp) gene were presented by Chung et al. for HEBV. We performed phylogenetic analyses to compare these swine-derived sequences with sequences from all mosquito-derived viruses from which we could sequence the corresponding genome region (Figure 2, panel B). Comparison of swine-derived sequences with the phylogeny of mosquito-derived HEBV strains, constructed on the basis of the third conserved region of the RdRp (Figure 2, panel C), showed that the strains from South Korea fell within the phylogenetic diversity of HEBV strains identified in West Africa. Online Technical Appendix Figure 3 shows nucleotide- and amino acid-based alignments.

## Conclusions

Our results contrast with those of Chung et al. (9) for several possible reasons. First, the viruses infecting swine in South Korea may constitute variants of GOLV and HEBV that can infect vertebrates. The presence of an NSs protein in phleboviruses and orthobunyaviruses provides interferon resistance required to infect vertebrates efficiently (3,4). Because full genome sequences from swine viruses detected by Chung et al. are not available, we have no information on the presence of NS proteins in these viruses. Furthermore, our detection assays might have failed to detect variant viruses. However, our RT-PCR assays have been shown to detect variant viruses, have been validated for sensitivity ( $\approx 100$  viral genome copies per mL in liquid specimens), and provide high

specificity by probe detection (5,6). A concern regarding the results of Chung et al. is the use of RT-PCR assays based on SYBR Green (Thermo Fisher Scientific, Lithuania) product detection, which, from our experience, is prone to yield non-specific results because no probe is used in this assay. Nevertheless, RT-PCR products in Chung et al. have been confirmed by sequencing. Some sequences presented by these researchers contained stop codons in the HEBV RdRp and the GOLV glycoprotein precursor genes, making it unlikely that these sequences represent replicating viruses. Besides technical explanations, these sequences could represent viral genome fragments integrated in genomes of organisms, such as insects, that are eaten by pigs in the region. Integration of RNA virids derived from flaviviruses into the host genome has been described in insects (11). Testing food eaten by swine for insect DNA or viral RNA could yield insight. In addition, we may have collected serum when no active virus infections occurred in tested animals. However, past infections would have been shown by antibody tests. Because bunyaviruses from all vertebrate-infecting genera induce antibodies against the nucleoprotein (12–14), we are confident about our choice of antigen in our assays. Chung et al. presented no serologic results to support virus detections (9).

Several technical issues in the study by Chung et al. should be clarified further. First, RNA concentration in tissue, as determined by RT-PCR, did not correlate with the success of probe-based immunohistochemistry in several organ samples (9). Second, supernatants from the virus isolate from South Korea showed high cytopathogenic activity in cell culture ( $10^3$ – $10^5$  cytopathogenic units/mL) but low levels of concomitant viral RNA by RT-PCR. Because no antigen detection in cells was attempted, the cytopathogenic effect could have been caused by any other virus blindly isolated. One of the most infectious and deadly



**Figure 2.** Maximum-likelihood phylogenetic analyses of Gouléako virus (GOLV) and Herbert virus (HEBV) strains from mosquitoes in Côte d'Ivoire, 2004, and Ghana, 2011, and virus strains detected by Chung et al. (9) in pigs in South Korea. A) Analysis of the glycoprotein precursor gene of GOLV strains identified in mosquitoes collected in Côte d'Ivoire and Ghana and of strains detected in swine in South Korea. Sequences originating from swine are shown in bold. B) Analysis of the RNA-dependent RNA polymerase gene of HEBV strains from mosquitoes and swine. Sequences originating from swine are shown in bold. C) Analysis of all identified HEBV strains found in mosquitoes. HEBV strains used for phylogenetic analyses in panel B are shown in bold. GOLV strains F25M/CI/2004 and F26M/CI/2004 were found in male mosquitoes. Scale bars indicate nucleotide substitutions per position in the alignment.

swine pathogens, the porcine reproductive and respiratory syndrome virus (15), was co-detected in lung samples of dead pigs in South Korea (9).

The finding of genome fragments of GOLV and HEBV in swine in South Korea needs to be more fully explored. However, with no further independent proof of infection of swine or other vertebrates, HEBV and GOLV should not be considered epizootic pathogens or arboviruses.

### Acknowledgments

We thank Pascal Trippner for technical assistance and the Max Planck Society for providing field work assistance in Côte d'Ivoire.

The project was supported by the Deutsche Forschungsgemeinschaft (grant agreement no. DR 772/12-1) to C.D.

Dr. Junglen is a biologist and scientist at the Institute of Virology in Bonn, Germany. Her main research interests are the diversity, evolution, and spread of arthropod-associated viruses.

### References

- Elliott RM. Orthobunyaviruses: recent genetic and structural insights. *Nat Rev Microbiol.* 2014;12:673–85. <http://dx.doi.org/10.1038/nrmicro3332>
- Elliott RM, Brennan B. Emerging phleboviruses. *Curr Opin Virol.* 2014;5:50–7. <http://dx.doi.org/10.1016/j.coviro.2014.01.011>
- Bridgen A, Weber F, Fazakerley JK, Elliott RM. Bunyamwera bunyavirus nonstructural protein NSs is a nonessential gene product that contributes to viral pathogenesis. *Proc Natl Acad Sci U S A.* 2001;98:664–9. <http://dx.doi.org/10.1073/pnas.98.2.664>
- Bouloy M, Janzen C, Vialat P, Khun H, Pavlovic J, Huerre M, et al. Genetic evidence for an interferon-antagonistic function of Rift Valley fever virus nonstructural protein NSs. *J Virol.* 2001;75:1371–7. <http://dx.doi.org/10.1128/JVI.75.3.1371-1377.2001>
- Marklewitz M, Handrick S, Grasse W, Kurth A, Lukashev A, Drosten C, et al. Gouléako virus isolated from West African mosquitoes constitutes a proposed novel genus in the family Bunyaviridae. *J Virol.* 2011;85:9227–34. <http://dx.doi.org/10.1128/JVI.00230-11>
- Marklewitz M, Zirkel F, Rwego IB, Heidemann H, Trippner P, Kurth A, et al. Discovery of a unique novel clade of mosquito-associated bunyaviruses. *J Virol.* 2013;87:12850–65. <http://dx.doi.org/10.1128/JVI.01862-13>
- Auguste AJ, Carrington CV, Forrester NL, Popov VL, Guzman H, Widen SG, et al. Characterization of a novel *Negevirus* and a novel *Bunyavirus* isolated from *Culex* (*Culex*) *declarator* mosquitoes in Trinidad. *J Gen Virol.* 2014;95:481–5. <http://dx.doi.org/10.1099/vir.0.058412-0>
- Marklewitz M, Zirkel F, Kurth A, Drosten C, Junglen S. Evolutionary and phenotypic analysis of live virus isolates suggests arthropod origin of a pathogenic RNA virus family. *Proc Natl Acad Sci U S A.* 2015;112:7536–41. <http://dx.doi.org/10.1073/pnas.1502036112>

9. Chung HC, Nguyen VG, Goede D, Park CH, Kim AR, Moon HJ, et al. Gouléako and Herbert viruses in pigs, Republic of Korea, 2013. *Emerg Infect Dis.* 2014;20:2072–5. <http://dx.doi.org/10.3201/eid2012.131742>
10. Meyer B, Muller MA, Corman VM, Reusken CB, Ritz D, Godeke GJ, et al. Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. *Emerg Infect Dis.* 2014;20:552–9. <http://dx.doi.org/10.3201/eid2004.131746>
11. Crochu S, Cook S, Attoui H, Charrel RN, De Chesse R, Belhouchet M, et al. Sequences of flavivirus-related RNA viruses persist in DNA form integrated in the genome of *Aedes* spp. mosquitoes. *J Gen Virol.* 2004;85:1971–80. <http://dx.doi.org/10.1099/vir.0.79850-0>
12. Lazutka J, Zvirbliene A, Dalgediene I, Petraityte-Burneikiene R, Spakova A, Sereika V, et al. Generation of recombinant Schmallenberg virus nucleocapsid protein in yeast and development of virus-specific monoclonal antibodies. *J Immunol Res.* 2014;2014:160316. <http://dx.doi.org/10.1155/2014/160316> Epub 2014 May 29.
13. Ergunay K, Kocak Tufan Z, Bulut C, Kinikli S, Demiroz AP, Ozkul A. Antibody responses and viral load in patients with Crimean-Congo hemorrhagic fever: a comprehensive analysis during the early stages of the infection. *Diagn Microbiol Infect Dis.* 2014;79:31–6. <http://dx.doi.org/10.1016/j.diagmicrobio.2013.12.015>
14. Williams R, Ellis CE, Smith SJ, Potgieter CA, Wallace D, Mareledwane VE, et al. Validation of an IgM antibody capture ELISA based on a recombinant nucleoprotein for identification of domestic ruminants infected with Rift Valley fever virus. *J Virol Methods.* 2011;177:140–6. <http://dx.doi.org/10.1016/j.jviromet.2011.07.011>
15. Chand RJ, Tribble BR, Rowland RR. Pathogenesis of porcine reproductive and respiratory syndrome virus. *Curr Opin Virol.* 2012;2:256–63. <http://dx.doi.org/10.1016/j.coviro.2012.02.002>

Address for correspondence: Sandra Junglen, Institute of Virology, University of Bonn Medical Center, Sigmund-Freud Str 25, 53127 Bonn, Germany; email: [junglen@virology-bonn.de](mailto:junglen@virology-bonn.de)

**SEARCH PAST ISSUES OF EID  
AT [WWWNC.CDC.GOV/EID](http://WWWNC.CDC.GOV/EID)**

# 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 Geneious (Biomatters, Auckland, 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 15µL of porcine serum samples mixed with 55µL 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'-GCTCTAGATCACTTGTCATCGTCGTCCTTGTAGTCACCAGCTTCCATCAGTTTTCCGGCCGC), HEBV-N-BamHI-F (5'-CGGGATCCGCCACCATGGCTACCAATTTTGAATTCAATGATAAC), and HEBV-N-FLAG-C-SphI-R (5'-ACATGCATGCTCACTTGTCATCGTCGTCCTTGTAGTCACCAGCTTGAGGCCATATTGTTGATCAGTG). 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 c-terminal 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-phenylindole).

## References

1. Marklewitz M, Handrick S, Grasse W, Kurth A, Lukashev A, Drosten C. Gouléako virus isolated from West African mosquitoes constitutes a proposed novel genus in the family Bunyaviridae. *J Virol.* 2011;85:9227–34. <http://dx.doi.org/10.1128/JVI.00230-11>
2. Marklewitz M, Zirkel F, Rwego IB, Heidemann H, Trippner P, Kurth A. Discovery of a unique novel clade of mosquito-associated bunyaviruses. *J Virol.* 2013;87:12850–65. <http://dx.doi.org/10.1128/JVI.01862-13>
3. Meyer B, Müller MA, Corman VM, Reusken CB, Ritz D, Godeke GJ, et al. Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. *Emerg Infect Dis.* 2014;20:552–9 <http://dx.doi.org/10.3201/eid2004.131746>.
4. Chung HC, Nguyen VG, Goede D, Park CH, Kim AR, Moon HJ, et al. Gouléako and Herbert viruses in pigs, Republic of Korea, 2013. *Emerg Infect Dis.* 2014;20:2072–5. <http://dx.doi.org/10.3201/eid2012.131742>

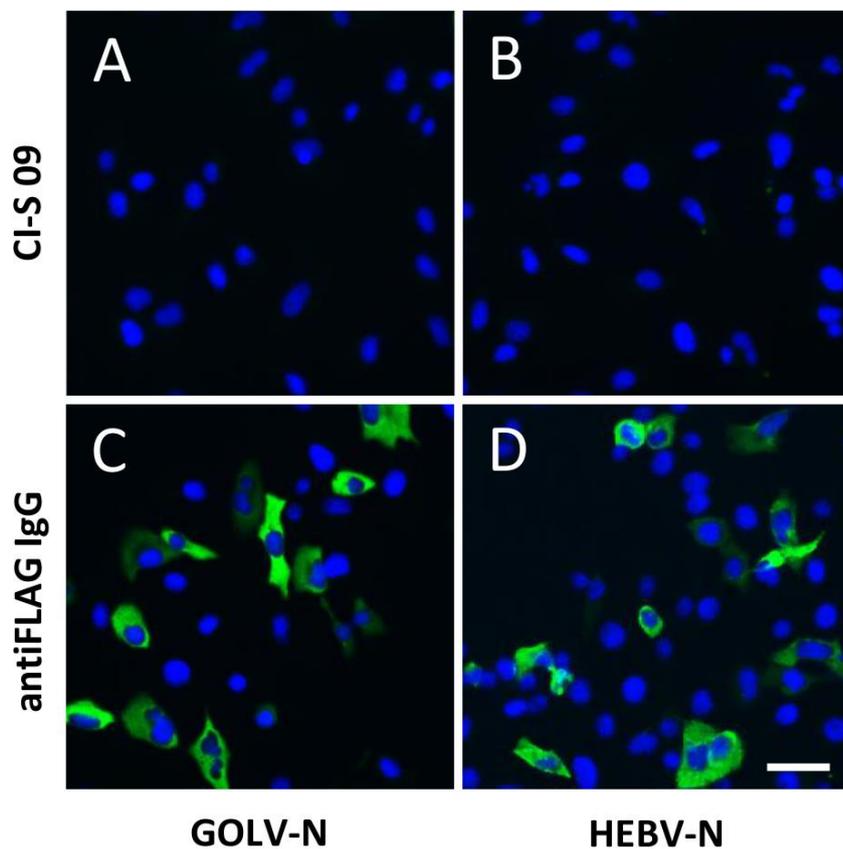
**Technical Appendix Table.** Porcine serum samples tested for infection with GOLV and HEBV by using real-time reverse transcription PCR and rIFA, in Côte d'Ivoire and Ghana, 2008–2011\*

Sample ID	Sex	Age, mo†	Origin	Year	rIFA		Viral RNA	
					GOLV	HEBV	GOLV	HEBV
CI-S 01	Male	12	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 02	Female	12	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 03	Female	12	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 04	Male	11	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 05	Female	9	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 06	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 07	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 08	Male	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 09	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 10	Female	6	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 11	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 12	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 13	Female	12	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 14	Male	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 15	Female	8	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 16	Female	4	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 17	Female	4	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 18	Female	2	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 19	Male	2	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 20	Female	4	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 21	Male	4	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 22	Female	2	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 23	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 24	Female	2	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 25	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 26	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 27	Female	3	Gouléako, CI	2008	Neg	Neg	Neg	Neg
CI-S 28	Female	5	Gouléako, CI	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

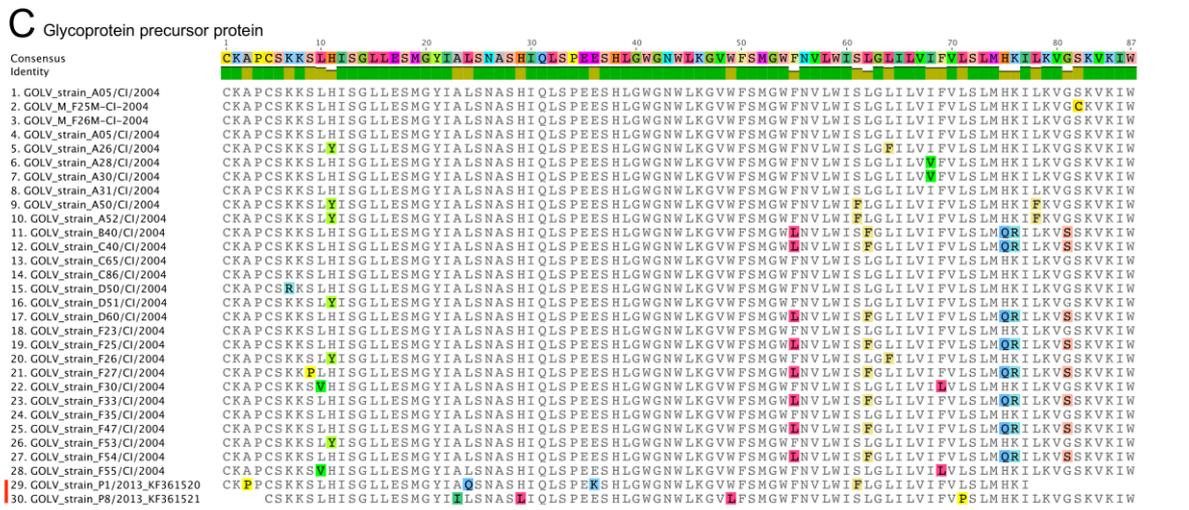
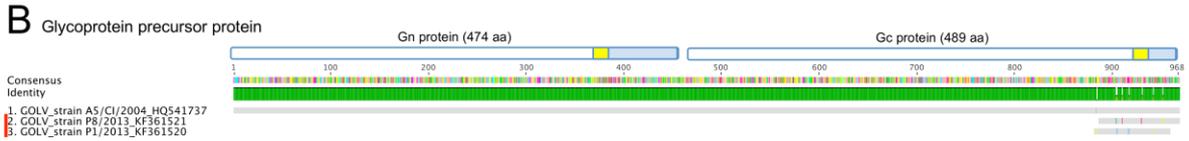
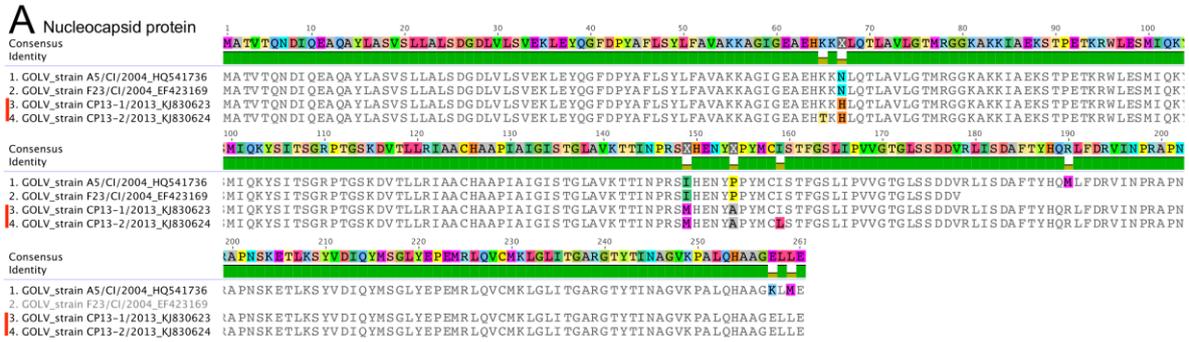
Sample ID	Sex	Age, mo†	Origin	Year	rIFA		Viral RNA	
					GOLV	HEBV	GOLV	HEBV
GH-S 15	Male	6	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 16	Female	1.5	Sokoban New Town, GH	2011	Neg	Neg	Neg	Neg
GH-S 17	Female	6	Sokoban New Town, GH	2011	Neg	Neg	Neg	Neg
GH-S 18	Male	1.5	Sokoban New Town, GH	2011	Neg	Neg	Neg	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	Neg	Neg	Neg	Neg
GH-S 21	Female	8	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 22	Male	6	Dompoase, GH	2011	Neg	Neg	Neg	Neg
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	Neg	Neg	Neg	Neg
GH-S 27	Female	6	Dompoase, GH	2011	Neg	Neg	Neg	Neg
GH-S 28	Female	8	Dompoase, GH	2011	Neg	Neg	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	Neg	Neg	Neg	Neg
GH-S 35	Male	5	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 36	Male	5	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 37	Female	5	Akropong, GH	2011	Neg	Neg	Neg	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	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 41	Male	6	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 42	Male	6	Akropong 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	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 47	Female	5	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 48	Male	5	Akropong, GH	2011	Neg	Neg	Neg	Neg
GH-S 49	Female	5	Akropong, GH	2011	Neg	Neg	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
GH-S 54	Male	7	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 55	Female	7	Akropong 2, GH	2011	Neg	Neg	Neg	Neg
GH-S 56	Female	7	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	Neg	Neg	Neg	Neg
GH-S 60	Male	5	Essienimpong, GH	2011	Neg	Neg	Neg	Neg
GH-S 61	Male	5	Essienimpong, GH	2011	Neg	Neg	Neg	Neg
GH-S 62	Male	4	Akropong 2, GH	2011	Neg	Neg	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	Neg	Neg	Neg	Neg
GH-S 67	Male	5	Essienimpong, GH	2011	Neg	Neg	Neg	Neg
GH-S 68	Female	5	Essienimpong, GH	2011	Neg	Neg	Neg	Neg
GH-S 69	Female	5	Essienimpong, GH	2011	Neg	Neg	Neg	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	Neg	Neg	Neg	Neg
GH-S 74	Female	8	Onwe, GH	2011	Neg	Neg	Neg	Neg
GH-S 75	Female	8	Onwe, GH	2011	Neg	Neg	Neg	Neg
GH-S 76	Male	8	Onwe, GH	2011	Neg	Neg	Neg	Neg
GH-S 77	Male	8	Onwe, GH	2011	Neg	Neg	Neg	Neg
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	Neg	Neg	Neg	Neg
GH-S 81	Female	5	Onwe, GH	2011	Neg	Neg	Neg	Neg
GH-S 82	Female	5	Onwe, GH	2011	Neg	Neg	Neg	Neg
GH-S 83	Female	7	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg
GH-S 84	Female	7	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	Neg	Neg	Neg	Neg
GH-S 89	Female	5	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg
GH-S 90	Female	5	Ejisu Krapa, GH	2011	Neg	Neg	Neg	Neg

Sample ID	Sex	Age, mo†	Origin	Year	rIFA		Viral RNA	
					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'Ivoire; GH, Ghana; GOLV, Gouléako virus; HEBV, Herbert virus; Neg, Negative results.  
†Age is age of pig from which serum sample was collected.

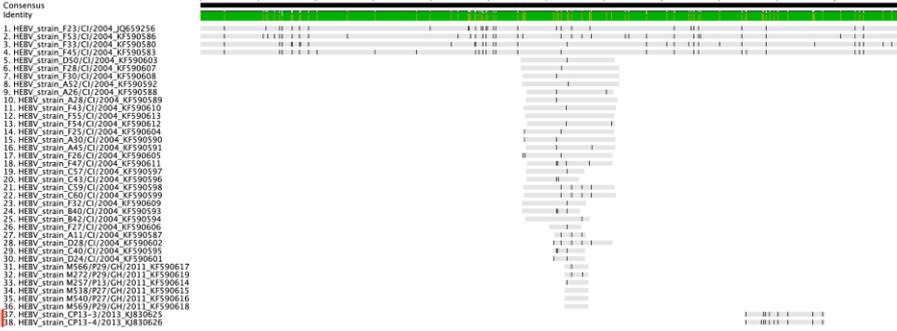


**Technical Appendix Figure 1.** Immunofluorescence patterns for antibodies against Gouléako virus (GOLV) and Herbert virus (HEBV) in serum samples from swine, Côte d'Ivoire (CI), 2008, and Ghana, 2011. Figure shows representative results from 1 pig (labeled CI-S 09) from which serum was tested against 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  $\mu$ m. All photographs were taken at equivalent exposure settings.

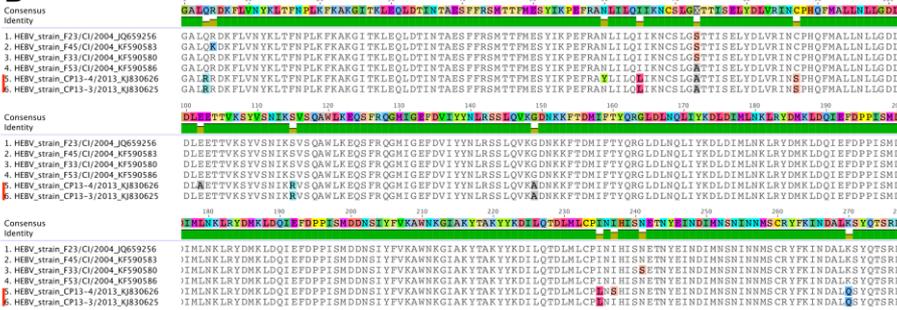


**Technical Appendix Figure 2.** Alignment of Gouléako virus (GOLV) strains from mosquitoes originating from Côte d'Ivoire and Ghana and swine sampled in Korea. A) Alignment of the GOLV nucleocapsid proteins; B) alignment of complete GOLV glycoprotein precursor protein from mosquito and of protein fragments 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).

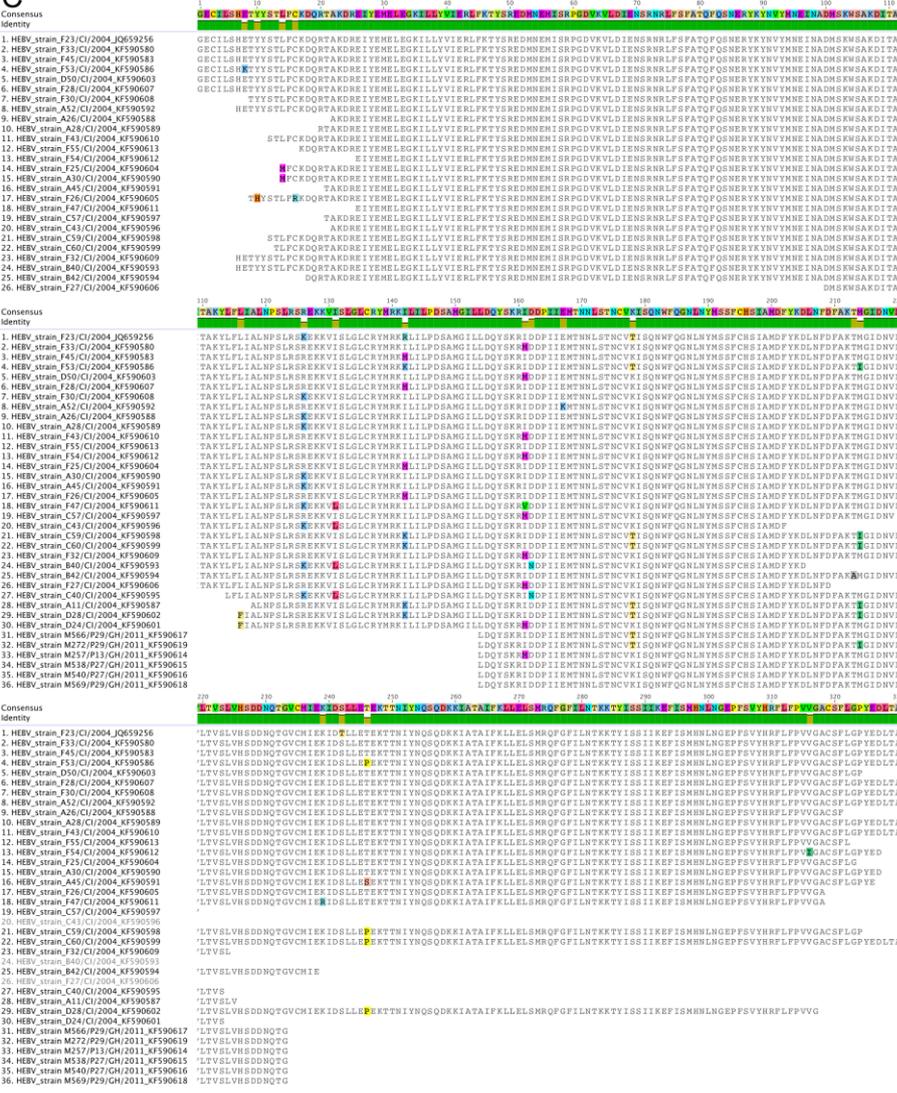
A



B



C



**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.