

Beyond surveillance, strategies for future vaccination of livestock and humans will benefit from robust epidemiologic data on RVFV and CCHFV to efficiently use resources across sub-Saharan Africa. Serologic tests relying on tag-free protein production, alternative tags, and careful validation of histidine-tagged antigens for specificity are mandatory for use of antibody tests in malaria-endemic regions.

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References

1. Tinto B, Quellec J, Cêtre-Sossah C, Dicko A, Salinas S, Simonin Y. Rift Valley fever in West Africa: a zoonotic disease with multiple socio-economic consequences. *One Health*. 2023;17:100583. <https://doi.org/10.1016/j.onehlt.2023.100583>
2. Temur AI, Kuhn JH, Pecor DB, Apanaskevich DA, Keshtkar-Jahromi M. Epidemiology of Crimean-Congo hemorrhagic fever (CCHF) in Africa – underestimated for decades. *Am J Trop Med Hyg*. 2021;104:1978–90. <https://doi.org/10.4269/ajtmh.20-1413>
3. Hawman DW, Feldmann H. Crimean-Congo haemorrhagic fever virus. *Nat Rev Microbiol*. 2023;21:463–77. <https://doi.org/10.1038/s41579-023-00871-9>
4. Petrova V, Kristiansen P, Norheim G, Yimer SA. Rift valley fever: diagnostic challenges and investment needs for vaccine development. *BMJ Glob Health*. 2020;5:e002694. <https://doi.org/10.1136/bmjgh-2020-002694>
5. Houessou SO, Dossa LH, Diogo RVC, Houinato M, Buerkert A, Schlecht E. Change and continuity in traditional cattle farming systems of West African Coast countries: a case study from Benin. *Agric Syst*. 2019;168:112–22. <https://doi.org/10.1016/j.agsy.2018.11.003>
6. Yadouleton A, Sander AL, Moreira-Soto A, Tchiboza C, Hounkanrin G, Badou Y, et al. Limited specificity of serologic tests for SARS-CoV-2 antibody detection, Benin. *Emerg Infect Dis*. 2021;27:233–7. <https://doi.org/10.3201/eid2701.203281>
7. Piche-Ovares M, García MP, Moreira-Soto A, Figueroa-Romero MD, Merino-Sarmiento NS, Marcelo-Nique AL, et al. Rio Mamore hantavirus endemicity, Peruvian Amazon, 2020. *Emerg Infect Dis*. 2024;30:2532–43. <https://doi.org/10.3201/eid3012.240249>
8. Ho MF, Baker J, Lee N, Luchavez J, Arie F, Nhem S, et al. Circulating antibodies against *Plasmodium falciparum* histidine-rich proteins 2 interfere with antigen detection by rapid diagnostic tests. *Malar J*. 2014;13:480. <https://doi.org/10.1186/1475-2875-13-480>
9. Debeljak N, Feldman L, Davis KL, Komel R, Sytkowski AJ. Variability in the immunodetection of His-tagged recombinant proteins. *Anal Biochem*. 2006;359:216–23. <https://doi.org/10.1016/j.ab.2006.09.017>
10. Boko KC, Ibrahim AI, Akpo Y, Alladassi ME, Aguidissou NO, Zoclanclounon AR, et al. Sero-prevalence of Rift Valley fever and brucellosis in cattle of northern Benin, West Africa. *Am J Anim Vet Sci*. 2022;17:267–73. <https://doi.org/10.3844/ajavsp.2022.267.273>

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Complete Genome Analysis of African Swine Fever Virus Isolated from Wild Boar, India, 2021

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Complete genome analysis of African swine fever virus isolated from a wild boar in Mizoram, India, revealed ~99% nucleotide identity with those of domestic pig origin but with unique mutations. A One Health approach toward food security necessitates awareness among veterinary and public health professionals on virus evolution and domestic–wild pig transmission.

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African swine fever (ASF) is a devastating disease affecting pigs, with death rates reaching 100%. Wild boars (*Sus scrofa*), warthogs (*Phacochoerus aethiopicus*), and bushpigs (*Potamochoerus porcus*) can act as asymptomatic carriers, contributing to virus persistence in a sylvatic cycle (1). Soft ticks of the genus *Ornithodoros* further complicate ASF epidemiology. The disease is caused by ASF virus (ASFV), a large, double-stranded DNA virus belonging to the genus *Asfivirus* in the Asfarviridae family. The ASFV genome range is 171–193 kb, featuring inverted terminal repeats (ITRs) at both ends. Different ASFV genotypes are based on the 3' end of the *B646L* gene; genotype II predominates in Asia, Europe, Oceania, and the Americas. Recent emergence of novel recombinant genotype I/II strains in China and Vietnam (2,3) is of great concern.

ASF was first reported in India in 2020 after outbreaks in domestic pigs in northeastern states (4). Outbreaks in wild boars have been documented

in Assam, Karnataka, and Tamilnadu states (5,6). ASFVs circulating in India belong to genotype II and intergenic region (IGR) subcluster II. Complete genome sequencing of Indian ASFV isolates of domestic pig origin revealed unique mutations in the *MGF 360–11L*, *MGF 505–4R*, *K205R*, and *B263R* genes (7). We analyzed the complete genome of ASFVs isolated after ASF outbreaks affecting domestic pigs and wild boars in Mizoram, India, in August 2021.

We collected 40 samples from dead domestic pigs and those suspected of having ASF (Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/31/8/25-0083-App1.pdf>); 38 of those samples and a dead wild boar tested positive for ASF genome by quantitative PCR and were confirmed by virus isolation. We grew, processed for viral enrichment, and sequenced 1 ASFV isolate (MZ/21/PO-324) of wild boar origin and 1 isolate (MZ/21/PO-314) of domestic pig origin. We submitted nucleotide sequences to GenBank (accession nos. PV023909 and PV023910) (Appendix).

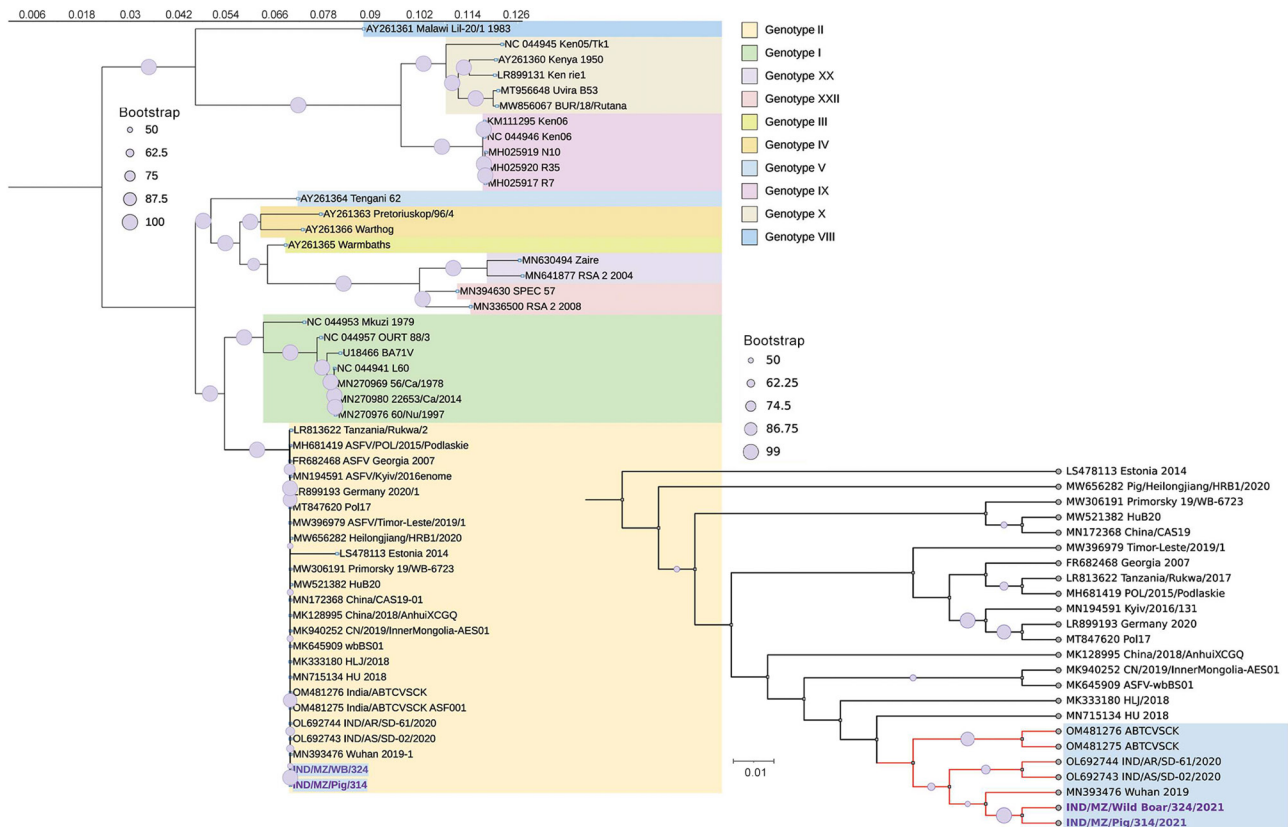


Figure 1. Phylogenetic tree generated from complete genome analysis of ASFV isolated from wild boar and domestic pig, India, 2021. The maximum-likelihood tree was MAFFT aligned (<https://mafft.cbrc.jp/alignment/software>) by using a general time-reversible plus gamma model in RAXMLGUI version 2.0 (<https://sourceforge.net/projects/raxmlgui>) and shows the relationship between ASFVs from India and other ASFVs including p72 genotype II. Enlarged area at bottom right shows detail of isolates from this study (bold text) and close reference sequences. GenBank accession numbers are provided. Scale bar indicates the number of expected substitutions per site (tree rooted at mid-point). ASFV, African swine fever virus.



Figure 2. Complete genome analysis of ASFV isolated from wild boar and domestic pig, India, 2021. A 50-nt deletion is observed in the *MGF-360–21R* gene of the ASFV isolate obtained from a wild boar in Mizoram, India, compared with ASFV isolates derived from domestic pigs in India. Bold isolates are from this study. GenBank accession numbers are provided. ASFV, African swine fever virus.

The wild boar ASFV genome comprised 190,489 bp with ITRs at the 5' end (1,597 bp) and 3' end (1,122 bp), whereas the ASFV genome from domestic pig measured 189,390 bp and had a 5' end ITR of 422 bp and 3' end ITR of 1,150 bp. Comparative analysis showed 99.93% nucleotide identity between those isolates (Appendix Figure 2). Phylogenetic analysis placed the ASFV in India within genotype II clade 2.2.2, alongside genotype II viruses reported during 2007–2023 across diverse regions. Within p72 genotype II viruses, isolates from India formed a distinct cluster with isolate ASFV/Wuhan/2019 (Figure 1). *EP402R* gene-based serogrouping confirmed isolates from India as part of serogroup 8, consistent with hemadsorption-positive viruses (Appendix Figure 3). There was an insertion of an additional tandem repeat sequence in the extragenic region between *I73R* and *I329L* (Appendix Figure 4), aligning with intergenic region II cluster isolates of genotype II. Central variable region analysis of the *B602L* gene indicated similarity to the Georgia 2007/1 central variable region I variant (8).

We observed multiple nucleotide insertions and deletions (Appendix Table 1) in the genome sequence of the wild boar isolate compared with isolate ASFV-Georgia/2007, leading to frame shift mutations in *DP60R* and *ASFV-GACD 190* genes, amino acid additions in *ASFV GACD-00300* and *ASFV GACD-00350* genes, and protein truncations in immune-modulatory genes, *MGF 110–7L*, *MGF 110–10L*, *MGF 110–14L*, *MGF 110–13Lb*, *I196L*, *B475L*, and *MGF 360–21R*, of which the last 3 mutations were unique to the wild boar isolate (Appendix). A 50-nt deletion in the *MGF 360–21R* gene resulted in a truncated protein of 327 aa. We did not observe that deletion in the ASFV

isolate of domestic pig origin, and the deletion was unique to the wild boar isolate reported in this study. Further analyses and multiple sequence alignment of *MGF-360–21R* gene of ASFV isolates obtained from wild boar, warthog, and domestic pigs across different countries revealed that the gene is particularly susceptible to mutations during replication in wild boars compared with domestic pigs (Figure 2) and causes truncations at the carboxyl terminus of the encoded protein. Those observations reflect the role of the *MGF-360–21R* gene in evolutionary adaptations of ASFV in wild boar populations.

A comparative analysis of genotype II ASFV revealed 20 single-nucleotide polymorphisms comprising 16 nonsynonymous and 4 synonymous mutations across 18 open reading frames (Appendix Figure 5). Key nonsynonymous mutations included K32E in *ASFV GACD 300*, P406L in *EP1242L*, R188K in *K205R*, and Q104H in *E199L*. The proteins encoded by *K205R* and *E199L* genes are noted to interact with host proteins, potentially activating cellular responses such as unfolded protein response and autophagy (9,10).

In conclusion, ASFV sequences from both hosts showed ~99% identity and highlighted transmission between domestic and wild pigs. However, we identified unique genetic variations in ASFVs isolated from wild boar, which may influence viral interactions with host cellular machinery. Our findings highlight the critical role of wild boars in ASF epidemiology and underscore the need for veterinary, wildlife and public health authorities to be aware of transmission dynamics between domestic and wild pigs and viral evolution, with implications for viral survival, immune modulation, and control strategies.

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References

1. Lubisi BA, Bastos AD, Dwarka RM, Vosloo W. Molecular epidemiology of African swine fever in East Africa. *Arch Virol*. 2005;150:2439–52. <https://doi.org/10.1007/s00705-005-0602-1>
2. Zhao D, Sun E, Huang L, Ding L, Zhu Y, Zhang J, et al. Highly lethal genotype I and II recombinant African swine fever viruses detected in pigs. *Nat Commun*. 2023;14:3096. <https://doi.org/10.1038/s41467-023-38868-w>
3. Lee K, Vu TTH, Yeom M, Nguyen VD, Than TT, Nguyen VT, et al. Molecular characterization of emerging recombinant African swine fever virus of genotype I and II in Vietnam, 2023. *Emerg Microbes Infect*. 2024;13:2404156. <https://doi.org/10.1080/22221751.2024.2404156>
4. Rajukumar K, Senthilkumar D, Venkatesh G, Singh F, Patil VP, Kombiah S, et al. Genetic characterization of African swine fever virus from domestic pigs in India. *Transbound Emerg Dis*. 2021;68:2687–92. <https://doi.org/10.1111/tbed.13986>
5. Buragohain L, Barman NN, Sen S, Bharali A, Dutta B, Choudhury B, et al. Transmission of African swine fever virus to the wild boars of Northeast India. *Vet Q*. 2023;43:1–10. <https://doi.org/10.1080/01652176.2023.2178689>
6. Sai Balaji KG, Karikalani M, Saravanan S, Mahajan S, Rajeshkumar K, Vaseem M, et al. High mortality in free-ranging wild boars associated with African swine fever virus in India. *Arch Virol*. 2024;169:137. <https://doi.org/10.1007/s00705-024-06065-9>
7. Senthilkumar D, Rajukumar K, Venkatesh G, Singh F, Tosh C, Kombiah S, et al. Complete genome analysis of African swine fever virus isolated from domestic pigs during the first ASF outbreaks in India. *Transbound Emerg Dis*. 2022;69:e2020–7. <https://doi.org/10.1111/tbed.14536>
8. Gallardo C, Casado N, Soler A, Djadjovski I, Krivko L, Madueño E, et al. A multi gene-approach genotyping method identifies 24 genetic clusters within the genotype II-European African swine fever viruses circulating from 2007 to 2022. *Front Vet Sci*. 2023;10:1112850. <https://doi.org/10.3389/fvets.2023.1112850>
9. Chen S, Zhang X, Nie Y, Li H, Chen W, Lin W, et al. African swine fever virus protein E199L promotes cell autophagy through the interaction of PYCR2. *Virol Sin*. 2021;36:196–206. <https://doi.org/10.1007/s12250-021-00375-x>
10. Wang Q, Zhou L, Wang J, Su D, Li D, Du Y, et al. African swine fever virus K205r induces ER stress and consequently activates autophagy and the NF-κB signaling pathway. *Viruses*. 2022;14:394. <https://doi.org/10.3390/v14020394>

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Influenza D Virus in Domestic and Stray Cats, Northern China, 2024

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Influenza D virus infects primarily cattle, but infrequent reports of infections in cats occur. We detected influenza D virus antibodies in 8 of 360 cats in northern China. Domestic cats showed higher susceptibility than strays. Our results suggest a previously overlooked aspect of epidemiology of this virus in companion animals.

Influenza D virus (IDV) is a single-stranded, negative-sense RNA virus belonging to the genus *Deletainfluenzavirus*, family Orthomyxoviridae, and was first isolated from pigs in 2011 (1,2). IDV infects primarily cattle, but studies have documented the virus globally in a diverse range of animals, including small

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