

African Swine Fever Virus p72 Genotype IX in Domestic Pigs, Congo, 2009

Carmina Gallardo, Raquel Anchuelo, Virginia Pelayo, Frédéric Poudevigne, Tati Leon, Jacques Nzoussi, Richard Bishop, Covadonga Pérez, Alejandro Soler, Raquel Nieto, Hilario Martín, and Marisa Arias

African swine fever virus p72 genotype IX, associated with outbreaks in eastern Africa, is cocirculating in the Republic of the Congo with West African genotype I. Data suggest that viruses from eastern Africa are moving into western Africa, increasing the threat of outbreaks caused by novel viruses in this region.

African swine fever (ASF) is a serious disease of domestic pigs caused by a DNA arbovirus (African swine fever virus [ASFV]) belonging to the family *Asfviridae* (1). Its highly contagious nature and ability to spread over long distances make it 1 of the most feared diseases of pigs; it causes devastating effects on pig production as manifested in the Caucasus since its introduction from southeastern Africa during 2007 (2). Considerable spread of ASF has been reported in western Africa during the past 20 years, and, except for in Côte d'Ivoire, the disease remains endemic (3). Because discernible ASFV serotypes are lacking, the field strains are grouped genetically by using sequencing of the C-terminus of the p72 protein, which discriminates 22 genotypes (4,5). Genotype I is historically associated with outbreaks in western Africa, whereas viruses from southern and eastern Africa have higher heterogeneity, with all 22 known genotypes having been recorded within the region (5–7).

The Republic of the Congo, located in western-central sub-Saharan Africa, shares borders with the Cabinda

enclave of Angola, the Democratic Republic of the Congo, Central African Republic, Cameroon, and Gabon. The last ASF outbreaks in Congo were reported to the World Organization for Animal Health (OIE) during 2003. Since then, the disease has been officially declared endemic but without quantitative data. Sampling and characterization of currently circulating field strains from this region of western-central Africa are needed to fully understand virus spread and maintenance. Such data will have implications for regional control in western Africa.

The Study

During August 2009, a United Nations Food and Agriculture Organization mission was undertaken with local counterparts in Congo to support development of an action plan to control ASF. A key factor in selecting the sites sampled was inclusion of the main pig-producing, marketing, and consuming areas, with a particular focus where suspected ASF outbreaks had been recently reported. From the survey conducted, 86 samples comprising serum (35 samples), whole blood (44 samples), and tissues (7 samples) were collected from 80 domestic pigs in the departments of Brazzaville, Pointe Noire, Kouilou, Bouenza, Niari, and Cuvette (Figure). These departments contain 80%–90% of the country's pig population. Samples also were collected from Pool in southeastern Congo, where a recent hemorrhagic disease outbreak, characterized by case-fatality rates of ≈80%, had been reported. Clinical material was sent to the European Union Reference Laboratory for African Swine Fever (Centro de Investigación en Sanidad Animal, Madrid, Spain) for confirmatory diagnosis and characterization of the ASFV strain(s) responsible for the outbreak(s).

Specific ASF antibodies were detected by using the OIE-prescribed assays (8) in 7 of 35 serum samples analyzed. All positive serum samples were from animals that had survived the ASF outbreaks in Bouenza, Niari, and Pointe Noire during 2008. For ASFV genome detection, OIE-prescribed PCRs (8) were performed on DNA extracted from 28 serum samples, 44 blood samples, and 7 tissue homogenates. A positive result was obtained in 17 (21%) of samples analyzed, indicating ASF in all the departments where the survey was conducted except for Kouilou.

Subsequently, 5 hemadsorbent Congo ASFVs (Table) were isolated in porcine peripheral blood macrophages (8). Viral DNA was extracted, and 3 different sets of primers were used for ASFV genotyping. A region of 478 bp at the C-terminal end of the p72 protein and the full-length sequence of *p54* gene were amplified by using primers p72U/D and 89/722, respectively (6). We compared the sequence from each of the p72 and p54 amplicons with homologous sequences representative of each previously

Author affiliations: Centro de Investigación en Sanidad Animal, Valdeolmos, Madrid, Spain (C. Gallardo, V. Pelayo, C. Pérez, A. Soler, R. Nieto, H. Martín, M. Arias); International Livestock Research Institute, Kabete, Nairobi, Kenya (R. Anchuelo, R. Bishop); United Nations Food and Agriculture Organization, Bamako, Mali (F. Poudevigne); and Conseiller à l'Élevage du Ministre de l'Agriculture et de l'Élevage, Brazzaville, Republic of the Congo (T. Leon, J. Nzoussi)

DOI: 10.3201/eid1708.101877

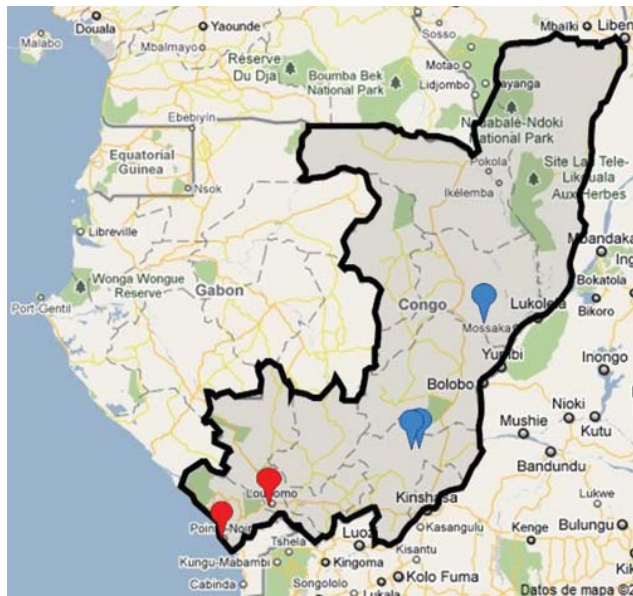


Figure. Republic of the Congo, showing location of the African swine fever virus isolates from eastern (blue) and western (red) Congo selected for genotyping.

described p72 and p54 genotype (5,6). A rooted minimum-evolution tree was constructed by using MEGA4.0 software and the p-distance nucleotide substitution model (9). Two different ASFV phylogenetic profiles were found in western and southeastern Congo. The phylogeny inferred for the ASFV isolates from eastern Congo (Con09/Pk45, Con09/Bzz020, Con09/Abo) placed them within p72 and p54 genotype IX with viruses collected from recent outbreaks in Kenya and Uganda during 2006–2007 (6,7). By contrast, ASFV isolates from western Congo (Con09/Ni16, Con09/PN003) clustered in p72 genotype I and p54 genotype Ic (6) comprising historical isolates from western Africa, such as Ang72 and Kat67, and the first ASFV isolated from Europe, the Portuguese Lisbon57 isolate (online Appendix Figure, www.cdc.gov/EID/content/17/8/101877-appF.htm).

To provide higher resolution, the tetrameric tandem amino acid repeat sequences within central variable region (CVR) of the *B602L* gene were analyzed (5–7,10). Primers CVR1 (5'-ACTTTGAAACAGGAAAC(AT)AATGATG-3') and CVR2 (5'-ATATTTTGTAATATGTGGGCTGCTG-3') were designed in this study selected from complete genome sequences of ASFV strains available in GenBank.

Under the same thermal cycling conditions used for full *p54* gene amplification, amplicons of 600–650 bp were generated from the isolates from eastern Congo, whereas the estimated size of the amplicons from western Congo was 500 bp (data not shown). Analysis of the tandem amino acid repeat sequences within the CVR demonstrated 3 different variants cocirculating in the country, 2 within the p72 type I virus genotype. The type of CVR sequence (AABNABTDBNAAAA) identified in the isolate from Pointe Noire (Con09/PN003) was identical to CVR subgroup XIII (11) that contains early isolates from Angola (Ang70, Ang72) and Portugal (Lis57). However, the Con09/Ni16, also classified within p72 and p54 genotypes I and Ic, showed a unique CVR sequence (AAAAAAAFAF) not previously described but most similar to viruses from Burundi and Kenya in CVR subgroup XXVI (7,11). As for p72 and p54 genotyping, isolates from eastern Congo were related to CVR subgroup XXIV, which contains isolates obtained during the 2006 and 2007 outbreaks in Uganda and Kenya (6,7).

Conclusions

We confirmed ASF in 5 of the 6 departments in which surveillance was conducted during August 2009 in Congo. Genotyping of 5 ASFV isolates from Congo resulted in identification of genetically distinct viruses circulating simultaneously in the country. In eastern districts of Congo, viruses were most genetically similar to those recovered from the outbreaks in Kenya and Uganda during 2006 and 2007. Therefore, genotype IX, associated with ASF outbreaks in

Table. African swine fever virus isolates selected for genotyping from domestic pigs after virus isolation for which nucleotide sequence was determined at 3 loci, Republic of the Congo, 2009*

Isolate	Sampling location			p72		p54		Central variable region	
	Locality	Dept	Province	Geno	GenBank	Geno	GenBank	Subgroup	GenBank
Con09/PN003	Tchimbamba	Ponte Noire	Southwestern	I	HQ645947	Ic	HQ645949	XIII	HQ645957
Con09/Ni16	Kikassa	Niari	Western	I	HQ645943	Ic	HQ645948	Related XXVI	HQ645953
Con09/Pk45	Ferme Champenoise Dimi, Igné	Pool	Southeastern	IX	HQ645944	IX	HQ645952	XXIV	HQ645954
Con09/Bzz020	Ferme Champenoise Dimi, Igné	Pool	Southeastern	IX	HQ645945	IX	HQ645950	XXIV	HQ645955
Con09/Abo	Ferme Ibovi	Abo, Cuvette	Eastern	IX	HQ645946	IX	HQ645951	XXIV	HQ645956

*Dept, department; geno, genotype; GenBank, GenBank accession number.

eastern Africa during the previous 10 years, also occurs in western Africa. By contrast, viruses in western Congo were similar to genotype I viruses that historically circulated in western Africa. Analyses of the CVR identified 2 discrete variants within genotype I. The Pointe Noire ASFV was identical to viruses isolated in neighboring Angola during the 1970s, indicating a prolonged persistence of this virus type in the region. By contrast, the ASFV isolated from Niari was related to variants circulating during the 1980s in Burundi. These results are consistent with recent transfer and dissemination of ASFV genotypes from eastern to western Africa. Broadening of our initial findings requires extending analyses to western-central Africa, particularly to countries such as Cameroon and the Democratic Republic of the Congo, and providing more in-depth analysis in Congo.

The apparent recent movement of a virus genotype previously associated with virulent ASF in eastern Africa to western Africa, where the viruses have hitherto always been classified in p72 genotype I, indicates the possibility of future outbreaks of disease caused by novel viruses in western Africa. The reality of this scenario recently was demonstrated in the Caucasus region. Our study confirms the continuing spread of ASFV.

Acknowledgments

We thank the Ministry of Agriculture of Republic of the Congo (Brazzaville) for its support and cooperation to carry out this work. We greatly appreciate the intellectual and practical contributions of our colleagues at Centro de Investigación en Sanidad Animal and International Livestock Research Institute.

This work was partly supported under ASFRISK (Evaluating and Controlling the Risk of African Swine Fever in the EU) project (DG-Research, EC, 7FP Grant Agreement) KBBE211691, the European Union Reference Laboratory for African Swine Fever (DG SANCO EC [European Commission's Directorate General for Health and Consumer Policy]), and by the grant ILRI2003-001 funded by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spanish Ministry of Science and Innovation.

Dr Gallardo is the laboratory research coordinator of the European Reference Laboratory for African Swine Fever at the

Centro de Investigación en Sanidad Animal. Her research focuses mainly on molecular characterization and diagnosis of ASFV.

References

- Dixon LK, Escribano JM, Martins C, Rock DL, Salas ML, Wilkinson PJ. *Asfarviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desseberger U, Ball LA, editors. *Virus taxonomy*, VIIIth report of the ICTV. London (UK): Elsevier/Academic Press; 2005. p. 135–43.
- Food and Agriculture Organization. EMPRES Watch: African swine fever in the Caucasus (April 2008) [cited 2009 Jul 31]. [ftp://ftp.fao.org/docrep/fao/011/aj214e/aj214e00.pdf](http://ftp.fao.org/docrep/fao/011/aj214e/aj214e00.pdf)
- Penrith ML. African swine fever. *Onderstepoort J Vet Res*. 2009;76:91–5.
- Bastos ADS, Penrith ML, Cruciere C, Edrich JL, Hutchings G, Roger F, et al. Genotyping field strains of African swine fever virus by partial p72 gene characterisation. *Arch Virol*. 2003;148:693–706. doi:10.1007/s00705-002-0946-8
- Boshoff CI, Bastos AD, Gerber LJ, Vosloo W. Genetic characterisation of African swine fever viruses from outbreaks in southern Africa (1973–1999). *Vet Microbiol*. 2007;121:45–55. doi:10.1016/j.vetmic.2006.11.007
- Gallardo C, Mwaengo DM, Macharia JM, Arias M, Taracha EA, Soler A, et al. Enhanced discrimination of African swine fever virus isolates through nucleotide sequencing of the p54, p72, and pB602L (CVR) genes. *Virus Genes*. 2009;38:85–95. doi:10.1007/s11262-008-0293-2
- Gallardo C, Okoth E, Pelayo V, Anchuelo A, Martín E, Simón A, et al. African swine fever viruses (ASFV) with two different genotypes, both of which occur in domestic pigs, are associated with ticks and adult warthogs, respectively, at a single geographical site. *J Gen Virol*. 2011;92(Pt 2):432–44.
- World Organization for Animals. *Manual of diagnostic tests and vaccines for terrestrial animals (mammals, birds and bees)*. Chapter 2.8.1. Paris (France): The Organization; 2008. p. 1069–82.
- Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol*. 2007;24:1596–9. doi:10.1093/molbev/msm092
- Owolodun OA, Bastos AD, Antiabong JF, Ogedengbe ME, Ekong PS, Yakubu B. Molecular characterisation of African swine fever viruses from Nigeria (2003–2006) recovers multiple virus variants and reaffirms CVR epidemiological utility. *Virus Genes*. 2010;41:361–8. doi:10.1007/s11262-009-0444-0
- Nix RJ, Gallardo C, Hutchings G, Blanco E, Dixon LK. Molecular epidemiology of African swine fever virus studied by analysis of four variable genome regions. *Arch Virol*. 2006;151:2475–94. doi:10.1007/s00705-006-0794-z

Address for correspondence: Carmina Gallardo, Centro de Investigación en Sanidad Animal (CISA-INIA), Ctra Algete el Casar s/n. Valdeolmos, Madrid, Spain; email: gallardo@inia.es



Now in PubMed Central

Emerging Infectious Diseases current and past content now in the National Library of Medicine's digital archive.