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Towards rational design of vaccines against African swine fever in Eastern and southern Africa: Correlation of viral genome differences with virulence and analysis of viral and target cell transcription and protein expression

country/countries	Eastern and southern Africa (mainly Uganda and Kenya)
funding agency	Federal Ministry of Food and Agriculture - BMEL
project management	Federal Office for Agriculture and Food – BLE
project coordinator	Dr. Walter Fuchs Friedrich-Loeffler-Institut Federal Research Institute for Animal Health
project partner(s)	
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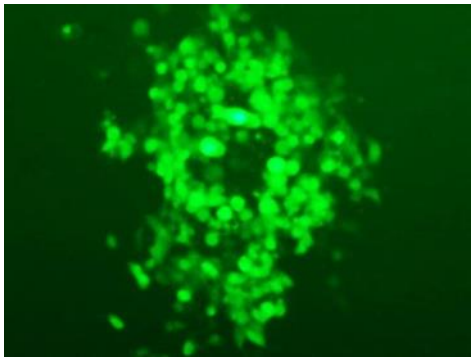
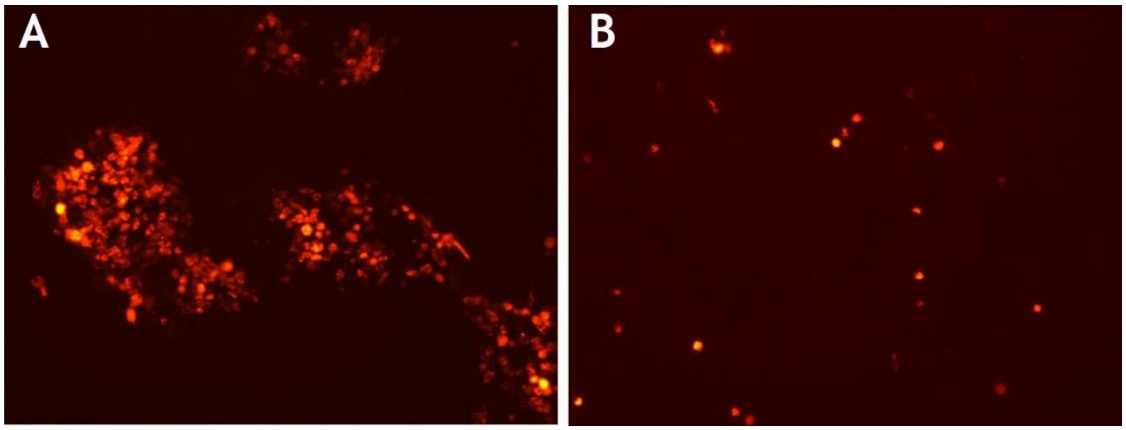
key words	African swine fever, live virus vaccines, vectored vaccines, resistant pigs

background	In many African countries the importance of pig breeding for nutrition and economy has increased substantially during the last decades. However, this development is inhibited by various animal diseases, among which African swine fever (ASF) is particularly problematic. While the infection is usually fatal in domestic pigs, the soft tick-borne African swine fever virus (ASFV) causes only mild symptoms in wart hogs and bush pigs, and is therefore widely distributed in these species, and frequently transmitted to their domestic cognates. Although laboratory experiments suggest that vaccination against ASF should be possible in principle, development of a feasible vaccine was not successful up to now.
objective	For development of potential vaccines virulence factors of ASFV should be deleted and immunogenic ASFV proteins should be expressed in viral vectors. Since ASFV isolates exhibit different virulence and considerable difference at DNA and protein levels, several viruses of different genotypes, which are currently relevant in Africa, should be compared using next generation sequencing and mass spectrometry, respectively. This could reveal a correlation between genetic markers and virulence. To verify the biological relevance of conspicuous genes, they had to be mutated by genetic engineering of cell culture adapted ASFV strains. As soon as isogenic groups of virulent parental ASF viruses and recombinants with potential virulence gene mutations were available, their replication *in vitro* and their pathogenicity *in vivo* had to be comparatively investigated. As far as possible, protective efficacy should be evaluated by subsequent challenge infections with virulent ASFV. Furthermore, abundant structural proteins of ASFV should be expressed in an attenuated pseudorabies vaccine strain (PrV-Bartha), and the protective efficacy of the obtained vector constructs in swine should be investigated. In a different approach, the CRISPR/Cas9 system was used to inhibit ASFV replication in cell culture by targeting single or multiple essential virus genes, which are conserved in different African and Eurasian genotypes. Final goal of these studies is the generation of ASF-resistant domestic pigs.
results	ASFV mutants were generated by transfection of a permissive wild boar lung cell line (WSL) with recombination plasmids, and subsequent infection with ASFV wild type virus. For enhancement of efficiency the deleted virus genes were cleaved by CRIPSR/Cas9, and replaced by transiently inserted reporter genes for fluorescent proteins (eGFP) and/or other selectable markers (CD4). Using these methods, five *in vitro* nonessential genes of a Kenyan genotype IX ASFV isolate could be deleted up to now: A104R, E165R (dUTPase), EP402R (CD2v), K196L (thymidine kinase), and K145R. From an Armenian genotpype II virus the KP177R (p22) und 285L were also deleted. The obtained mutants exhibited at best minor replication defects in cell culture. The deletion of essential ASFV genes was not yet successful. Using BAC- and CRISPR/Cas9 technology we were able to express 15 different ASFV proteins abundantly in the PrV vaccine strain Bartha, including the capsid protein p72, and surface proteins p12, p22, p30 and p54. The residual virulence of the ASFV mutants, and the protective efficacy of the attenuated ASP viruses and vector constructs remains to be tested.

	Furthermore, recombinant WSL cells were generated, which stably expressed Cas9 nuclease and single guide RNAs (sgRNAs) against essential ASFV genes. While genotype-specific sgRNAs against CP204L (p30) selectively inhibited replication of either ASFV Armenia (II) or Kenya (IX), expression of an O61R (p12) specific sgRNA almost completely abolished replication of any ASFV isolate. First experiments further indicated that these systems can be also stably expressed in transgenic swine, and therefore, might be able to confer resistance against ASFV.
Recommendations	The improved methods for generation of ASFV recombinants and vector constructs should be also established in the affected African countries to permit production and validation of affordable genotype-specific vaccines.
photos	 Pig farm with ASFV-infected animal in Uganda  Virus plaque of a thymidine kinase gene-deleted and GFP-expressing mutant of ASFV Kenya on WSL cells  Virus spread of a RFP-expressing mutant of ASFV Kenya normal WSL cells (A), and inhibitory CRISPR/Cas9 cells (B).