Sustainable Animal Productivity for Livelihoods, Nutrition and Gender Inclusion (SAPLING)
Knowledge Report - Progress Report

1. Report title
Laboratory reagent generation for the advancement of African swine fever Research

2. Participating Members (please add more sections for additional partners if needed)	
Partner 1	
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3. Background information

African swine fever (ASF) is a highly contagious hemorrhagic viral disease of domestic and wild pigs and causes serious economic and production loses. The causative agent of ASF, African swine fever virus (ASFV), is endemic in sub-Saharan Africa and leads to high mortality rates in domestic pigs. In the absence of vaccines or effective treatments use of biosecurity protocols remains the only available means of disease prevention. Thus, ASF hampers the livelihoods and food security of smallholder farmers. Besides, the ASFV virus is now present in Asia, Europe and America. The availability of an efficient ASF vaccine would contribute to the control of the disease and help to improve the pig production and livelihoods of smallholder farmers in low- and middle-income countries (LMIC), where biosecurity measures are more difficult to implement. To aid the delivery to remote areas, the vaccine should be stable without the need for cold chain. New vaccine delivery technologies, such as the use of nanoparticle antigen or mRNA to deliver subunit vaccines could have advantages compared to more 'traditional' delivery systems, such as soluble antigen, and of course with no risk of introducing new ASFV strains to an area.

A product from the livestock health flagship of the CGIAR Research Program on Livestock is a series of laboratory Standard Operating Procedures (SOPs) for the advancement of ASF vaccine and diagnostics research, such as ImmunoPeroxidase Monolayer Assay, enzyme-linked immunosorbent assay (ELISA), virus neutralization assay, among others. However, the commercial reagents, for example, to the p30 and p54 antigens of ASFV, to perform the SOPs are very expensive and not always available in LMIC.

Another product from the livestock health flagship and the International Veterinary Vaccinology Network project, IVVN001, is an ASFV protein plasmid expression library consisting of each gene of the ASFV-Kenya-1033 ASFV. This library is being used to generate a detailed immunological map of antibody responses to infection in pigs and other suids, including those that are tolerant to disease.

Expression plasmids can simply be used to generate gene specific regents such as those used in the SOPs described above. For example, rabbit sera were raised following immunization of animals with plasmids containing either the CP204L and E183L gene, which encode p30 and p54 antigens of ASFV, respectively. Sera generated by these animals have proved to be extremely useful to standardize SOPs with these 'home-made' reagents. This type of product (sera and cells) is what is referred to as reagents in this

report. However, it is desirable to develop such assays using reagents developed from pigs as these are the target animals for research.

4. Planned work and budget

The objective of this project was to develop pig-based reagents to the p30 and p54 ASFV antigens and use them to enhance the current laboratory SOPs while reducing their cost and availability in LMIC. The accessibility of reagents developed in the same species is a huge step forward to standardize, validate and eventually have Good Laboratory Practices for *in vitro* protocols in the ILRI laboratories. Secondly, pigs are larger animals than rabbits and the quantity of sera, cells or other body components relevant for immune responses to be used as reagents is proportionally larger.

The project plan is to immunize two groups of domestic pigs (Landrace x Duroc x Large White breed) consisting of two animals per group. After the primary dose, animals will be boosted two times with 100 μg of protein per dose (either p30 or p54) formulated with the Montanide ISA206VG (Seppic) adjuvant and injected intramuscularly 21 days apart (see visual representation below). The study was approved by the Institutional Animal Care and Use Committee (IACUC), reference number IACUC2022-28.

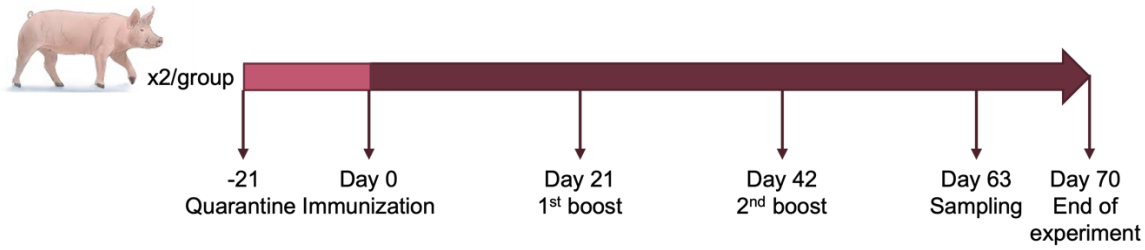


Figure 1. Schematic representation of the animal experiment under IACUC2022-28, where domestic pigs where immunized with African swine fever proteins pCP204L/p30 or pE183L/p54.

Production of the p30 protein will be outsourced from Algenex Ltd. (Spain) and the p54 protein will be expressed, purified and Quality Controlled (QC) at the ILRI facilities.

Animal samples will be collected before every dose of antigens and at day 63 (21 days after the last boost) and antibody titres assessed. Cellular responses will be assessed at day 63 using an IFNγ-ELISpot assay. If results are satisfactory the four animals will be euthanized at day 70 and blood for sera and isolation of blood cells will be collected to maximum possible amounts. Spleen cells will be also collected during post-mortem investigations. The sera, blood and spleen cells will constitute the pig reagents to advance the research in ASF.

Budget for 2022

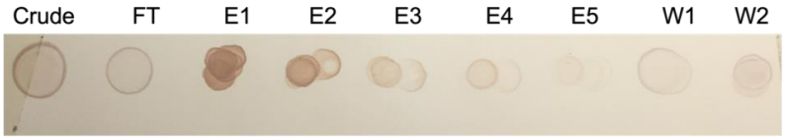
Experimental work (Farm)		$10,000
Laboratory reagents ($10,000/year)		$5,000
Personnel	10% FTE RA	$3,500
	10%FTE Scientist	$10,600
		TOTAL $28,900

5. Progress in the planned work

In this section we report on results up to the end of year 2022.

Two mg of highly purified p30 protein was obtained from Algenex Ltd. (Spain), in enough quantities for animal experiment and to perform *in vitro* work. The protein was quality controlled by Algenex before shipment and at the ILRI facilities once delivered using SDS-PAGE (data not shown). The protein was stored frozen for future use.

The p54 protein was expressed in transfected HEK293 mammalian cells with plasmid pE183L and cell lysates used for p54 protein purification using His-Trap Columns (Cytiva) as described by the manufacturer.



FT: flowthrough, E: Elution, W: Wash

Figure 2. Dot Blot of the different fractions generated during purification of pE183L/p54. Primary antibody used was anti-flag (p54 is tagged at the Ct with Flag tag) from GeneScript at 1:1,000 and the secondary goat anti-mouse:HRP at 1:1,000 (Merk). The reaction was developed using 3-3'-Diaminobenzidine (DAB) as a substrate.

Domestic pigs negative for ASFV antibodies and the presence of virus by PCR were immunized as described above. The animal experiment followed the planned schedule, and no incidences were reported by the farm staff or institutional veterinarian.

Table 1. Summary of IACUC2022-28 pig immunogenicity study groups.

Group	Animal ID	Immunogen	Dose	Number doses
Group 1	PT022	p30	100 µg/dose	3
	PT044	p30	100 µg/dose	3
Group 2	PT058	p54	100 µg/dose	3
	PT068	p54	100 µg/dose	3

Animals were bled before each immunization (Figure 1) and antibody titres against the antigens p30 and p54 were assessed by means of dot blot.

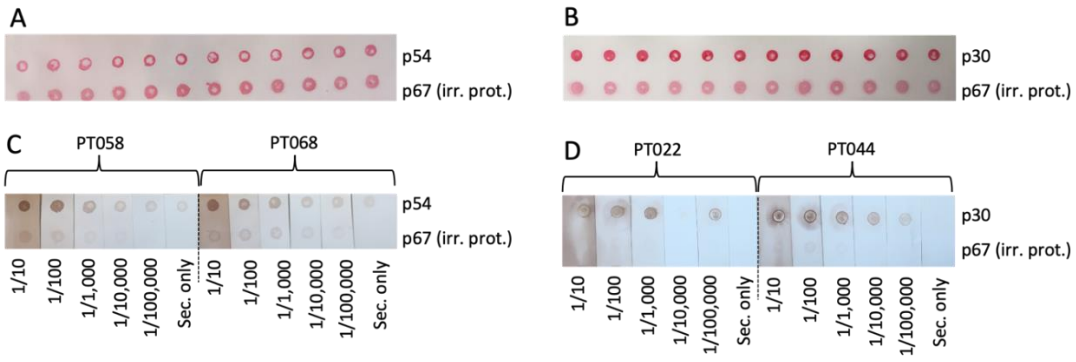


Figure 3. Immunoblots to estimate the antigen-specific antibody titres at day 42 (three weeks after the second dose) in domestic pig sera immunized with protein. (A) and (B) Punceau Staining to assess presence of protein in the membrane for pE183L/p54 or pCP204L/p30, respectively. Three proteins were included in each nitrocellulose membrane, the target purified protein (either pE183L/p54 or pCP204L/p30), an irrelevant protein used as negative control (p67C from *Theileria parva*) and crude material from GFP protein expression in HEK293 to assess the antibody reactivity to the expression cell lysate. Dot blot to assess the antigen-specific antibody titres in the pig serum samples against pE183L/p54 (C) or pCP204L/p30 (D), using a 10-fold serial dilution of sera. A secondary antibody control (rabbit anti-pig:HRP, Merk) was included for every set of membranes.

At day 42 signal was detected at up to 1/10,000 dilution of sera (antibodies) for both animals (PT058 and PT068) immunized with p54, whereas antigen-specific antibodies to p30 are still detectable at 1/100,000 dilution for both animals (PT022 and PT044). These antibody titres are very satisfactory and allow us to take the decision to go ahead with the sampling at day 63 for both sera and cells for IFNγ-ELISpot.

6. Next steps

On 1 January 2023, the animal experiment will be at day 52 in the experiment. At day 63 (three weeks after the last boost) animals will be bled again and antibody titres assessed by means of immunoblots and ELISA to determine the final antibody response. An IFNγ-ELISpot will also be performed at day 63 to assess the cellular response to the virus Kenya1033 (Genotype IX), a strain of special interest for ILRI ASF research.

At day 70 in the experiment, four weeks after the last boost, animals will be euthanized since they cannot go back to the meat chain. Animals will be bled to exhaustion for both serum and blood cells. Post-mortem investigations will take place and spleens extracted. Sera and cells will be kept frozen and available to all ASF research lines at ILRI.

The generation of such reagents for its use in the laboratories will facilitate the development of new techniques and standardize the existing ones at Good Laboratory Practices level. They also reduce the budget burden of the commercial products and the difficulty to get such reagents in LMIC.

Citation: Lacasta, A. 2022. *Laboratory reagent generation for the advancement of African swine fever research.* Nairobi, Kenya: ILRI.