Field sampling report – Pig skin biopsy and fibroblast culture trial

Moses Ogugo, Christine Kamidi Muhonja, Gad Milton Owido and Christian Keambou Tiambo

International Livestock Research Institute

January 2023

ILRI
INTERNATIONAL LIVESTOCK RESEARCH INSTITUTE

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ILRI thanks all donors and organizations which globally support its work through their contributions to the CGIAR Trust Fund

Editing, design and layout—ILRI Editorial and Publishing Services, Addis Ababa, Ethiopia.

Cover photo: ILRI/Gad Milton

Citation: Ogugo, M., Muhonja, C.K., Owido, G.M. and Tiambo, C.K. 2023. *Field sampling report – Pig skin biopsy and fibroblast culture trial:* Nairobi, Kenya: ILRI

Patron: Professor Peter C. Doherty AC, FAA, FRS
Animal scientist, Nobel Prize Laureate for Physiology or Medicine–1996

Box 30709, Nairobi 00100
Kenya
Phone +254 20 422 3000
Fax +254 20 422 3001
Email ilri-kenya@cgiar.org

ilri.org
better lives through livestock

ILRI is a CGIAR research centre

Box 5689, Addis Ababa, Ethiopia
Phone +251 11 617 2000
Fax +251 11 667 6923
Email ilri-ethiopia@cgiar.org

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Contents

Background 1

Objectives 2

Conclusions and way forward 5

References 6

Field sampling information

Project title: Conservation of tropical indigenous Suidae genetic resources using the induced pluripotent stem cells (iPSC) derived from somatic cells

1st Phase:

Sampling period: From 23 November to 27 November 2022

Locations: Busia County - Busia town, North Teso, South Teso, and Funyula

2nd Phase:

Sampling period: From 29 November to 4 December 2022

Locations: Migori County - Muhuru Bay, Sori and Ndhiwa.

Host institutions: Centre for Tropical Livestock Genetics and Health (CTLGH)/LiveGene ILRI Lab3, Reproductive Technology lab

Supported by: This research is being implemented by CGIAR researchers from the International Livestock Research Institute (ILRI) in close partnership with Veterinary Services from Kenya. We would like to thank all funders who supported this research through their contributions to the CGIAR Trust Fund: https://www.cgiar.org/funders/

Field sampling team: Christine Muhonja, Gad Milton Owido and Moses Ogugo

Participating institutions:

ILRI, Directorate of Veterinary Services-Kenya

Background

The rationale/justification for the project

Suidae is a family of artiodactyl mammals which are commonly called pigs, hogs, or boars. This family comprises the *Sus* (including the domestic pig *Sus scrofa*), babirusas and warthogs.

Local Suidae in Africa, including the domestic pig are underutilized, even though they provide a large contribution to the genetic diversity of the domestic stock and their genetic variability has been related to high adaptation to harsh environments and meat quality characteristics. Be they domesticated or wild, they are often part of the cultural heritage of local and national communities, having an important socio-economic value in their geographic regions.

Human activities such as deforestation, persecution and bushmeat hunting constitute substantial threats leading to the decline or extinctions of these animals. Further, local pig farmers tend to keep the herd sizes small to adequately meet the animals' nutrition needs, while most local pigs in western Kenya are reared in free range. This leads to small populations that are vulnerable to inbreeding and at risk if extinction. Hence the urgent need for their conservation.

The conservation of these locally adapted domestic and wild pig genetic resources using the most accessible biological material and non-invasive biotechnologies will increase the capability to respond to present and future needs of the global livestock production, and affordable and accessible biological material for further research on health threats like the African swine fever.

Stem cells (iPSCs) are undifferentiated stem cells characterized by the ability to differentiate into any cell type in the body. They can be obtained from any cell from the animal. Induced pluripotent stem cells (iPSCs) is a relatively new and rapidly developing technology in many fields of biology, including developmental anatomy and physiology, pathology, reproduction etc. The great potential of these cells for research and conservation lies on their self-renewing and pluripotent aptitudes, while attracting minimal ethical concerns. Protocols for iPSCs production have been developed for many domestic animal species (Scarfone et al. 2020). As compared to mesenchymal stem cells (MSCs) and embryonic stem cells (ESCs), iPSCs is less invasive and provides a more practical alternative to creating ESC-like cells in species where recovery of embryos or in vitro fertilization is difficult or not possible (Chow et al. 2017). iPSC technology, therefore, provides new strategies for the conservation of the genetics of any given mammalian species.

Porcine iPSCs (piPSCs) have been differentiated into several cell types for research purposes (Yang et al. 2013; Webb et al. 2017; Kim et al. 2019; Wei et al. 2020; Genovese et al. 2017; Ao et al. 2014; Gao et al. 2019; Kinoshita et al. 2021). Combining with the surrogate host technology, the iPSCs could be a revolutionizing technology for conservation and resuscitation of tropical pig breed without their genetic integrity being affected.

Objectives

To harness the potential of stem cell technologies for the conservation of local suidae (domestic pigs, ***Sus scrofa).***

Activities carried out

Harvesting pig skin

Animals were accessed in their natural environment, with the assistance of the community leaders and the local extension agents from the ministries of livestock. After cleaning with 70% ethanol, biopsies of fresh skin tissues from healthy animals were collected at the ear notch or back skin of the animal respecting the Institutional Animal Care and Use Committee (IACUC) approval from the International Livestock Research Institute (ILRI). These samples were then transported to the lab for single cell isolation and culture.

The field work in Busia County started on 23 November 2022 with the meeting with County Veterinary Directorate at 8am.

The choice of sites was done by Allan Ogendo, County Director of Veterinary Services. The Directorate also assigned the team local veterinary staff who assisted with selection of farmers.

We delivered a questionnaire to the farmers after seeking their consent to take the samples, took GPS coordinates in each sites all this was done using ODK.

The pigs were restrained using a stainless pig catcher. The site of skin biopsy was clean shaved then thoroughly sterilized using 70% ethanol. The site of biopsy was held using holding surgical forceps. It was then pulled up and an incision made below the forceps using a sterile scalpel blade. The incised part (3.5 mm) was sliced into small pieces and transferred to 15 ml falcon containing 6 ml of feeder + antibiotic +fungicide, and immediately transferred to cool box with cold ice packets. The other piece in 1.8 ml cryotube was snap frozen in liquid nitrogen. The samples were transferred each evening after sampling for storage in a 4°C fridge in the veterinary office. After three days all samples were moved mobile 4°C fridge and transported to ILRI Nairobi campus for laboratory processing.

The second phase of the work covered Migori County. The same protocol was used as in Busia County. The first day was a meeting with the County Director of Veterinary Services, Erick Were. We had a very robust discussion which did not only cover sampling of indigenous pigs. He believed we are also slowly losing indigenous chicken due to quick adoption of exotic chicken and introduction of improved 'kienyeji' (local) breeds. He is a breeder and has passion for conservation of indigenous livestock. The director selected for us sites which are keeping indigenous pigs. The sites selected were Muhuru Bay, Sori and Ndhiwa. In the field we were assisted by local veterinary staff in the selection of farmers.

We delivered a questionnaire to the farmers after seeking their consent to take the samples, and took GPS coordinates in each site using ODK.

Photo: Gad Milton / ILRI 2022

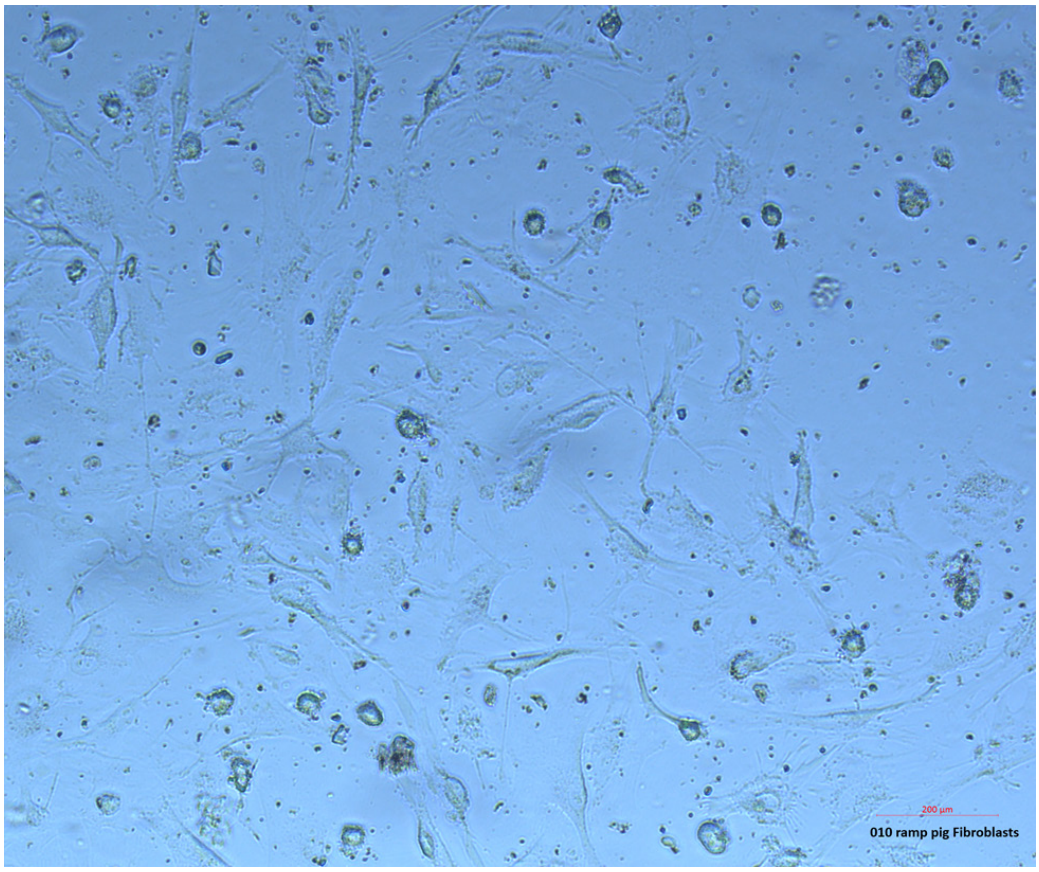
Table 1 summarizes the distribution of samples collected.

Table 1: Summary of pig skin biopsy samples collected in Busia and Migori counties.

County	Location	Number of samples
Busia County	Busia town	5
	North Teso	7
	South Teso	7
	Funyula	13
Migori County	Muhuru Bay	4
	Sori	4
	Ndhiwa	17

Cells isolation and tissue culture

The samples were cleaned using sterile PBS then crushed in the Laminar flow hood. Then digested in collagenase for 2 hours at 37°C. They were then passed through 70µm mesh cell strainer, and centrifuged. The cells were resuspended in DMEM with 10% FBS +1% antibiotic/antimycotic solution at 37°C with 5% CO2. This was done in 6 well plates.



Conclusions and way forward

We collected a total of 30 pig skin biopsies from Busia County and 30 skin biopsies from Migori County according to the protocol in a 15mls sterile falcon tube containing 6mls of feeder medium + antibiotic + anti-fungicide. We collected duplicate samples. One copy was snap frozen in liquid nitrogen.

We are processing the samples in the laboratory for the cultivation of fibroblast. We have uploaded the data in the server for SLICK WORK.

Growing cells were transferred to conical flask after day 16.

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