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Identification of a New Domestic Pig Cell Line for Growth of African Swine Fever Viruses for Vaccine Production in Uganda

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Abstract. In this study, a basis for the feasibility of a successful ASF vaccine design program using live attenuated vaccines was sought. One of the challenges to African swine fever (ASF) vaccine development is having a cell line that will provide commercial utility for vaccine production. We set out to address this problem by innovatively identifying possible cell lines from local domestic pigs in the country. Eight tissue types from ASF-negative animals were identified for incorporation into cell line development. These were degraded, incubated, and monitored for cell growth. One cell line, the SIR2-P, grew consistently and confluently and was tested for the ability to grow and isolate field ASF viruses. We report the isolation of ASF viruses in our laboratory for the first time. The P9C virus from Namayingo district exhibited the largest plaque sizes compared to the SQ517B virus from Mukono. The innovative identification of the SIR2-P cell line is proof of concept that newer species-specific cell lines can be developed in the Infectious Animal Disease Laboratory (IADL), and utilized to study other animal viruses like Porcine Reproductive and Respiratory Syndrome (PRRS) virus, Foot and mouth disease (FMD) virus, and Swine Influenza. Such cells can be used in vaccine production for other endemic diseases in Uganda. The SIR2-P cell line is currently at passage 65.

Keywords: Cell line; Isolation of African swine fever viruses; Live-attenuated vaccines.

Introduction

African swine fever (ASF) is a notifiable porcine disease caused by a double-stranded DNA virus, the African swine fever virus (ASFV). It is endemic in Uganda, with outbreaks happening every year, in a near-predictable fashion. ASF causes huge and sometimes irremediable production losses to mostly subsistent smallholder pig farmers in Uganda. Pig farmers usually never recover to continue with the piggery business after an outbreak. Although the disease is endemic, the country would desire to keep track of and control all ASF outbreaks, an undertaking that is hard to achieve financially and sustainably.

In this study, a basis for the feasibility of a successful ASF vaccine design control program using live attenuated vaccines was sought. One of the challenges to ASF vaccine development

is having a cell line that will provide commercial utility for vaccine production. We therefore sought to address this problem by identifying possible cell lines from local domestic pigs in Uganda, since it is common for such a resource to be very expensive to obtain from international vaccine producers or manufacturers. We therefore selected several ASF-negative tissues for inclusion in our experiments.

Methodology

Selection of Tissue Types

Following several cross-sectional field surveys involving the collection of suspected ASF-positive tissue (Ezinga, unpublished), we obtained several porcine tissues from several geographically-spread and distinct domestic pigs in Uganda. We selected 8 tissue types from negative animals for incorporation into development of a cell line (Table 1).

Table 1. Tissue types that were used for the cell extraction experiments

	Unique ID	Animal origin	Tissue type
1	KAP09	Porcine	Tonsil
2	SIR2	Porcine	Muscle
3	KAP13	Porcine	Lung
4	SIR4	Porcine	Muscle
5	AMU1	Porcine	Spleen
6	KAP10	Porcine	Lung
7	KAP05	Bovine	Lung
8	KWN07	Porcine	Lymph node

All field tissues are currently stored at -80^{o}C in NaLIRRI.

Approximately, 1 x 1 x1 cm^3 sized field tissues were aseptically debrided from original field tissues and carefully aliquoted into 2ml sterile cryovials.

Tissue degradation and incubation

Tissue aliquots were then homogenised and crushed using liquid nitrogen, followed by enzymatic degradation using 1 mL trypsin *(2000 U/G batch no. L250461806)* for 30 min. The cryovials were then centrifuged briefly at 2000 rpm for 5 mins, and the supernatants were transferred into 8 already prepared and well-labelled T25 cell culture flasks (Eppendorf AG). The supernatants were incubated under 5% CO_2 and at 37^{o}C using Eagle's Minimum Essential Medium (Lot# RNBJ1562), 10 % Fetal Calf Serum and 1% Penicillin/Streptomycin for at least 5 days.

Checking cell growth and adhesion

The T25 flasks were then checked for possibility of cell growth and confluency using a light microscope (Motic Light Microscope) at x20 and x40 magnification. Cells grew variedly and three cell lines were eventually selected for continued growth and passage. After three passages, one cell line grew consistently and confluently. This prompted testing of the new cell line for the growth and isolation of field viruses of African swine fever.

Isolation of African swine fever viruses on newly selected cells

Using previously confirmed ASFV-positive field viruses (Ezinga, unpublished) (Fig. 1), we infected and incubated for 5-7 days the newly-selected cell line with 3 matching virus supernatants derived from outbreaks in Namayingo (P9C), Bunyangabu (BUN05) and Mukono (SQ517B) districts during the 2019 – 2020 period. Viruses SOR36 and KAS04 from Soroti and Kasese, respectively, were infected and incubated on the new cell line too.

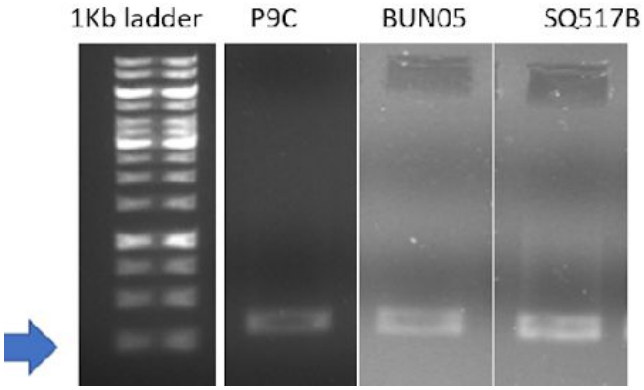

Figure 1. Positive identification using partial amplification of the p72 gene of ASFV.

P9C represents the amplification of an outbreak strain from Namayingo district (Eastern Uganda), BUN05 from Bunyangabu district (Western Uganda), and SQ517B from Mukono district (Central Uganda).

Results

Selection of the three cell lines

Using an inverted light microscope, 8 T25 flasks were observed, and cells were checked for growth and adhesion to the flasks. In three T25 flasks cells grew to at least over 60% confluence (Fig 2).

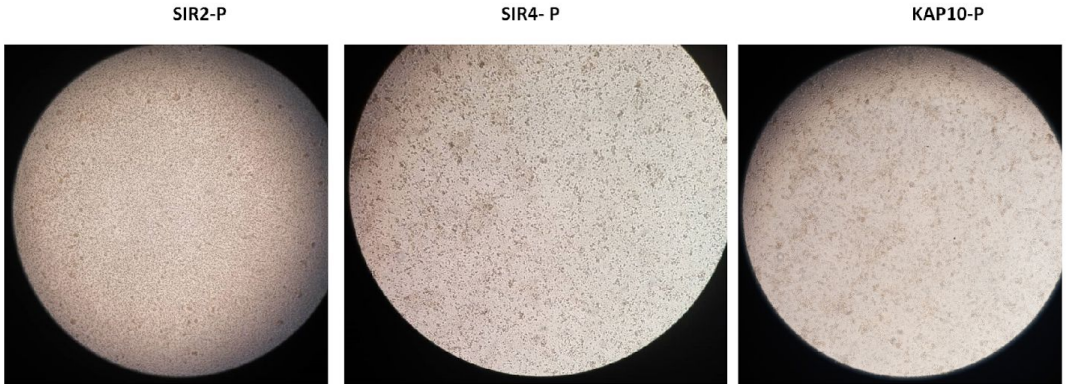

Figure 2. Confirmation of three cell lines – SIR2-P, SIR4-P, and KAP10-P all derived from domestic pigs observed at x20 magnification.

SIR2-P exhibited over 90% confluence and grew consistently across several passage cycles. In the remaining 5 T25 flasks, cells grew in isolation and were monitored for a few more days and did not continue to show the possibility of further growth. These were discarded and discontinued.

Continued passaging of selected cell lines to check for stability

We continued to split and incubate the three cell lines SIR2-P, SIR4-P, and KAP10-P up to three rounds. SIR-4 and KAP10-P showed varied confluence of utmost 50% and were disregarded (Fig 3).

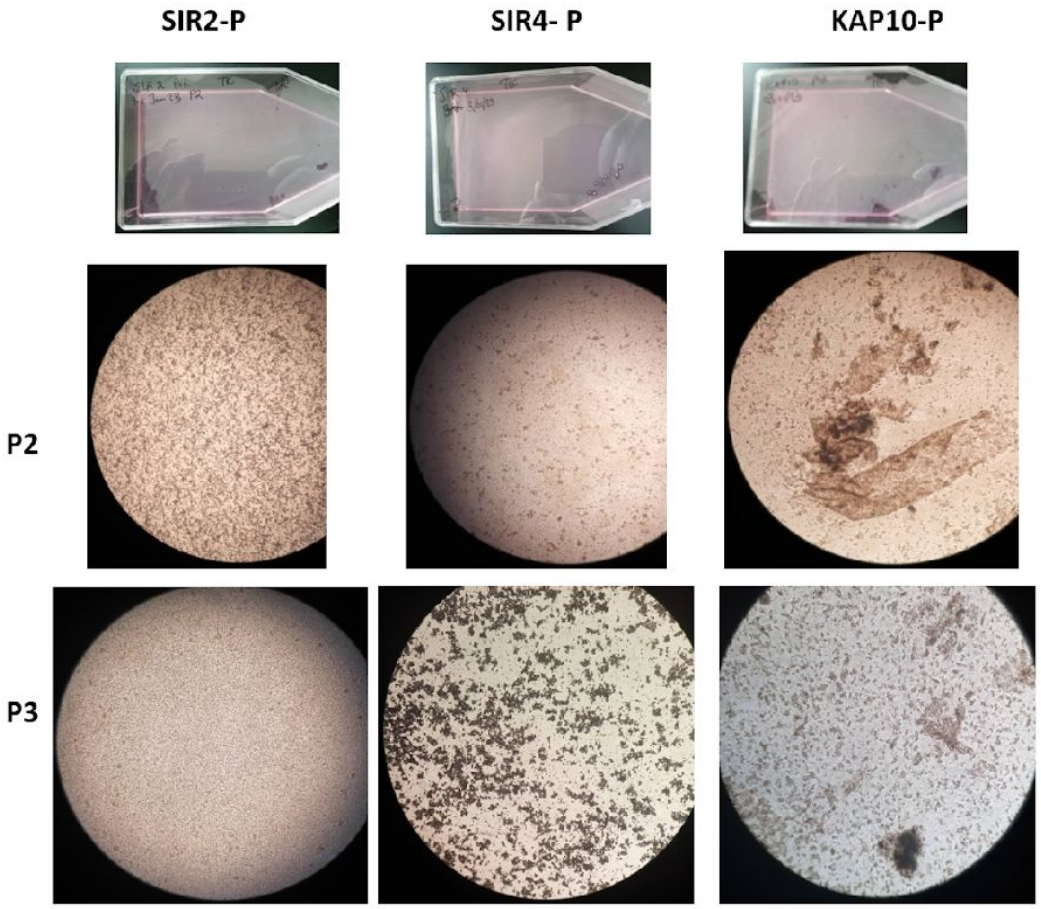

Figure 3. Continued passage of three cell lines to check for stability and survivability of seeded cell, X 20 magnification.

SIR2-P selection (at Passage 3)

After three passages, the SIR2-P cells appeared confluent and healthy and were selected as the new cell line for which to carry forward with several analyses.

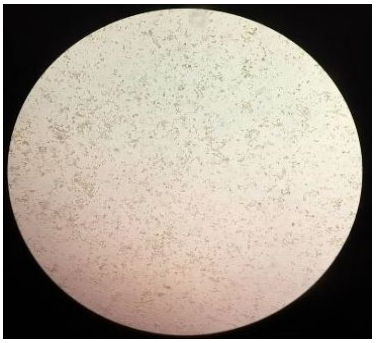

Figure 4. SIR2-P cells after the 3-passages observed at x20 magnification

Isolation of African swine fever viruses on the SIR2-P cell line

After 5-7 days of infection and incubation, 3 T25 flasks (Fig. 5) were analysed for virus growth on the SIR2-P cells, and virus plaques were identified in all three flasks.

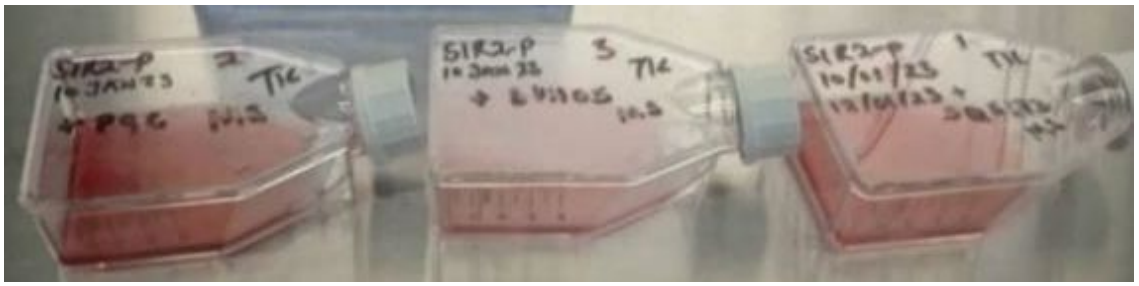

Figure 5. Infection of SIR2-P cells with ASFV-positive supernatants derived from positively identified field outbreaks

The viruses exhibited varied plaques sizes with P9C from Namayingo district displaying the largest plaques, and SQ5173 from Mukono having the smallest sized plaques as shown in Fig 6.

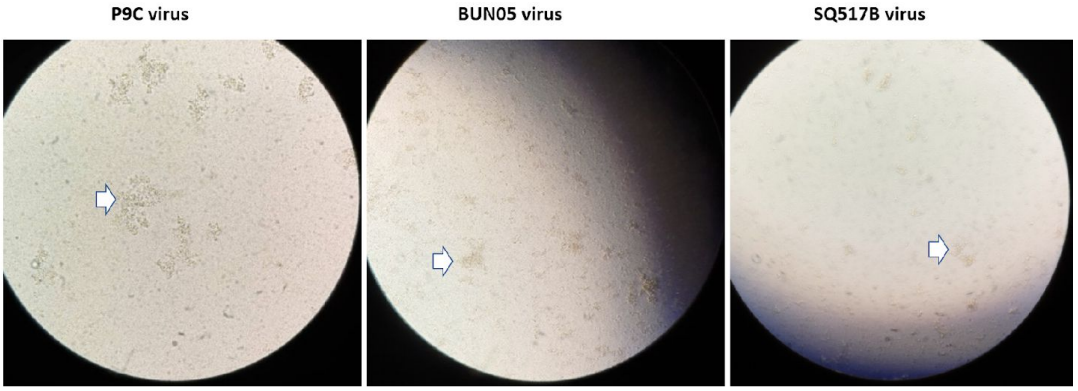

Figure 6. White arrows show ASFV plaques seen under light microscopy, at X40 magnification

The P9C virus from Namayingo district exhibited the largest plaque size, while SQ517B from Mukono showed variably smaller plaque sizes.

Aliquoting the SIR2-P domestic pig cell line for storage and vaccine development work

Several 2ml cryovials were labelled well and the SIR2-P cells were briefly centrifuged at 2000 rpm for 10 mins. The pellets were resuspended in MEM with 10% DMSO and cryo-preserved under absolute ethanol at -80^{o}C (Fig 7).

Figure 7. Cryo-preservation of SIR2-P cell line aliquots in the NaLIRRI cryo-bank for future reference

Discussion

African swine fever continues to be endemic in Africa (Gallardo et al., 2011, Yona et al., 2020) with a constant threat of spread to newer trans-continental boundaries where it has not existed before (Rahimi et al., 2010, Alkhamis et al., 2018, Schulz et al., 2020) (Chenais et al., 2015, Gallardo et al., 2015, Smith et al., 2019) (Sanchez-Cordon et al., 2019). Furthermore, ASF still has no licensed commercial ASF vaccine (Penrith et al., 2009), and it is known that within the current 24 genotypes (Bastos et al., 2003), there is no possibility of cross-protection, and conventional ASF virus genotyping may not distinguish between viruses of different virulence (Malogolovkin et al., 2015). The possibility of making ASFV vaccines has been shown (Arias et al., 2017, Gaudreault et al., 2019) (Sereda et al., 2020), for instance, a trial intra-genotypic ASF vaccine only offers homologous protection (O'Donnell et al.,2015, Sanchez-Cordon et al., 2020) (Sang et al.,2020). Similarly, it is known that for ASF, elevation of regulatory components of the immune system like regulatory T cells and IL-10 may inhibit effective protection (Sanchez-Cordon et al., 2020).

In Uganda two genotypes exist, for which genotype IX is the most prevalent in domestic pigs (Atuhaire et al., 2013, Kabuuka et al., 2014, Nantima et al., 2015, Chenais et al., 2017, Masembe et al., 2018, Onzere et al., 2018, Norbert et al., 2019). Given the endemicity and rebound nature of ASF outbreaks in Uganda (Muhangi et al., 2015, Penrithet al., 2021), we desired to have home-grown solutions to address the continued problem of ASF outbreaks. We attempted to design a live-attenuated vaccine (LAV) for the prevalent genotype IX ASF viruses in Uganda, since it has been shown that LAVs elicit and provide some levels of immune protection (O'Donnell et al., 2015, O'Donnell et al., 2017, Bosch-Camos et al., 2020, Gladue et al., 2020, Teklue et al., 2020) (Lopez et al.,2020 , Reis et al.,2020).

With the increasing technical ability in the country for vaccine design, and sometimes, the expensive cost coupled with the inability to acquire biological requirements for vaccine research

in Africa, we opted to inventively identify possible ways of growing cells in our laboratory (Sanchez et al.,2017, Portugal et al.,2020, Rai et al.,2020, Masujin et al.,2021, Meloni et al.,2022), and further use them to isolate ASF viruses for the first time in Uganda (Hurtado et al.,2010). Eventually, we desire to potentially use these cells as a precursors for virus production of live-attenuated vaccines (Hubner et al.,2019, Borca et al.,2020, Ramirez-Medina et al., 2022) (Keil et al., 2014) or attenuated strains of ASFV (Krug et al., 2015).

From our study, we categorically identified the SIR2-P cell line from a domestic pig that was ASF-negative. Cell lines are used to study and characterise viruses in well-laid out biosafe environments. This new cell line provides our laboratory with a set of new dimensions to study ASFV. We managed to show that the new cell line can be deplored for virus isolation, which is often a preserve of highly advanced laboratories in Africa. Since at our disposal was conventional PCR (partial amplification of the p72 gene of ASFV) as a confirmatory test, the observation of virus plaques under light microscopy needs to be backed up with indirect immunofluorescence antibody tests to mark-out the virus plaques. Subsequently after virus isolation, we investigated plaque sizes for viruses representing three broad regions in Uganda. From eastern Uganda, P9C and SOR36 viruses from Namayingo and Soroti districts, respectively, grew to comparable plaque sizes. From Western Uganda, BUN05 and KAS04, representing Bunyangabu and Kasese districts, respectively, showed varying plaque sizes. SQ517B from Mukono district, a representative of central Uganda was analysed and formed the larger plaque of all strains. It is worth noting that plaque-size differences from the ASFV strains warrants further in-vitro analyses for titer determination. Our new cell line accommodates this purpose very well.

To create live-attenuated ASFV vaccine candidates, transfection of permissive cells with the desired plasmids is essential. Our team will therefore attempt to transfect the SIR2-P cell line with deletion plasmids followed by superinfection of utmost three field viruses as determined by results from the in-vitro growth kinetics of the five isolated viruses. Upon evaluation of the three vaccine candidates, the SIR2-P cell line will be used for propagation of our vaccine candidates for production of trial batches.

Our group now seeks to serially passage the five isolated field ASF viruses and this will be followed by possible identification of an attenuated strain for further trial as a vaccine candidate.

The identification of the SIR2-P cell line is proof-of-concept that newer species-specific cell lines can be developed in NaLIRRI, and utilised to study important viruses affecting animals and further, be used in home-grown vaccine production of endemic diseases in Uganda.

Our work therefore brings to front a biological innovation of a cell line with the ability to improve research in Uganda and Africa at large through improved diagnosis through virus isolation, ability to conduct experiments that attenuate virulent viruses, and lastly, to provide a backbone for vaccine production at both small and large-scale in Uganda. Our new cell line will bring further use through studies involving molecular genetic characteristics and immune-pathogenic mechanisms of ASFV. The SIR2-P cell line continues to grow well and is currently at passage 42.

Conflict of Interest

The authors declare that they have no conflicts of interest in relation to this article.

Acknowledgement

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