NPB FINAL RESEARCH GRANT REPORT FORMAT

Project Title and NPB project identification number: NPB project #20-159 entitled "Evaluation of Lyophilization on efficacy of African swine fever vaccine candidate ASFV-G-ΔI177L

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Date Report Submitted4/14/2022

Industry Summary: Currently there is no commercialized vaccine for African swine fever(ASF). One of the most promising candidates to date is the highly effective and safe live attenuated vaccine candidate ASFV-G-ΔI177L, which is a recombinant virus that lacks the I177L gene. This candidate vaccine currently requires that the vaccine be kept frozen, which adds complexity to deliver the vaccine to areas that may not have the required freezing capabilities, such as rural areas in Africa or Asia, where ASF currently is causing outbreaks. A vaccine is an essential part of any eradication program. To overcome the limitation of cold storage, here we tested the ability of Lyophilization or freeze drying the vaccine, and the stability of the vaccine being stored at ambient or refrigeration temperatures. By using different combinations of stabilizing formulations, we have shown stability of a lyophilized version of ASFV-G-ΔI177L for up to two months. This is a major milestone in overcoming the required frozen storage of the ASFV-G-ΔI177L candidate vaccine.

Key Findings:

- Stabilizing buffers allow for the stable lyophilization of ASFV-G-ΔI177L
- Lyophilization of ASFV vaccines will help enable vaccination in rural areas where ASFV outbreaks are currently occurring
- Optimizing Stabilizing buffers increased stability of the vaccine from days to months at refrigeration or room temperature

Keywords: ASF, ASFV, African swine fever, Vaccine, Freeze-drying, Lyophilization

Scientific Abstract: African swine fever is currently causing outbreaks throughout Asia, Europe and Africa, and recently has been reported in the Dominican Republic and Haiti. Now ASF is in close proximity to the United States. Currently there are only experimental vaccines for ASF with the most promising one being a live attenuated vaccine ASFV-G-ΔI177L, that contains a deletion of the I177L gene. The initial preparation of this vaccine done in primary swine macrophages, was stored in liquid form at -70°C. This study was to evaluate the ability of the vaccine to be lyophilized, and the stability of the vaccine under different storage conditions.

Introduction:

African swine fever virus (ASFV) is a significant threat to agriculture in the United States. Since ASF has spread into Asia and Eastern Europe over the last decade, the concern for further spread and introduction into the U.S. has heightened. There are currently no commercial vaccines available to protect pigs against infection with ASFV and disease can result in devastating losses with up to 100% mortality. Of special concern is the highly pathogenic Georgia isolate, which is currently causing the current outbreaks all across Asia and Eastern Europe. Several characteristics of ASF such as the uncontrolled movement of wild boar and domestic swine create significant challenges for disease eradication. Thus, preventing the introduction of ASFV is a top priority for countries negative for the virus, such as the U.S.

We have recently discovered several new experimental live-attenuated vaccine candidates. One candidate ASFV-G-ΔI177L has several unique characteristics over other experimental vaccine candidates: i) 100% protection at doses as low as 10^2 HAD. ii) No residual virulence, including fever at 10^6 HAD. iii) Induces sterile immunity iv) Vaccine virus does not shed to unvaccinated animals. We are currently in the process of seeking commercial partners for this vaccine candidate. One aspect that is a concern for all live-attenuated vaccine candidates for ASF is that of stability, especially in areas of Asia and Africa, where the guarantee of a cold chain may not be possible.

One way that commercial vaccine producers increase the stability of vaccines is though lyophilization. This has been shown to be possible for many different viruses including poxviruses, closely related in its structure to ASFV. However, to date no one has determined the possibility of lyophilization of ASFV or ASFV live-attenuated vaccines.

Here we propose to try several different lyophilization formulations consisting of bovine serum albumin (BSA), l-glutamic acid (L-Glu), polyethylene glycol (PEG), and dextran (DEX) to test ASFV-G-ΔI177L for stability after lyophilization. We will test several different formulations and after lyophilization we will test for infectivity of the virus, by viral titrations. Successful formulations will then be tested for storage at different temperatures for set periods of time between 1 week and six months. The most stable formulation of ASFV-G-ΔI177L will be reconstituted and tested for vaccine efficacy in pigs against ASFV-Georgia.

Objectives:

The primary objectives are:

1. Test stability of ASFV-G-ΔI177L to be lyophilized
2. Compare different formulations for freeze drying and stability over time
3. Test the ability of lyophilized ASFV to vaccinate swine against ASFV-Georgia

Materials & Methods:

Cell culture and viruses

Primary cultures of blood-derived swine macrophages were performed using peripheral mononuclear adherent cells obtained by density gradients and adhesion to Primaria T25, 6- or 96-well dishes at a density of $5x10^6$ cells per ml and inoculated with an MOI of 0.01 of vaccine candidate ASFV-G-ΔI177L. Once the entire culture showed cytopathic effect (CPE) the culture was frozen, thawed and cellular debris was pelleted. This batch of ASFV-G-ΔI177L was aliquoted for use in the freeze-drying experiments. Titrations were done using the standard Reed and Munich technique.

Stabilizer Formulations

Formulations listed below using the ratios of stock vaccine to stabilizer formulation.

Batch one

Stabilizer Formulation		
	1:1 ratio	
F1	Sorbitol	10%
	sucrose	7%
	BSA	4%
F2	sorbitol	10%
	sucrose	7%
	glycine	4%
F3	Sorbitol	10%
	sucrose	7%
	FBS	9%
F4	Sorbitol	10%
	sucrose	7%
	trehalose	4%
F5	trehalose	4%
	FBS	9%
	Sorbitol	10%
F6 (control)	DMEM	

Batch 2:

Stabilizer Formulation 5:1 ratio		
F1	Skim milk	10%
	sucrose	5%
F2	skim milk	10%
	sorbitol	5%
F3	skim milk	10%
F4	skim milk	10%
F5	DMEM	
	sorbitol	10%
F6	DMEM	
	Sucrose	10%
F7	DMEM	
	sorbitol	10%
	sucrose	7%
	BSA	4%
F8	DMEM	
	sorbitol	10%
	sucrose	7%
	FBS	9%
F9	DMEM	
	BSA	4%
F10	sorbitol	10%
	sucrose	7%
	BSA	4%
F11	sorbitol	10%
	sucrose	7%
	FBS	9%
F12	trehalose	4%
	FBS	9%
	sorbitol	10%
F13	DMEM	
	skim milk	10%

Results:

Objective 1: Test stability of ASFV-G-ΔI177L to be lyophilized

Initially we lyophilized the vaccine candidate in culture media, and found that using this method we found that we were unable to find any measurable amount of our vaccine candidate surviving lyophilization when left at room temperature and a severe reduction in, we tested several different lyophilization techniques, all resulting in the same results.

Objective 2: Compare different formulations for freeze drying and stability over time

We then tested 6 different formulations listed as batch one in material and methods using a stock vaccine with a titer of 5.5 $log_{10}TCID_{50}$ The results were that all stabilizer formulations and RT storage for three weeks week were unable to stabilize the vaccine candidate(figure 1). When stored at refrigeration temperatures of $4^{o}C$ for three weeks. We did see an increase in stability of the lyophilized vaccine candidate formulation 3, however the decrease of about 3 logs in a short time

period when compared to the input vaccine was unacceptable.

Figure 1

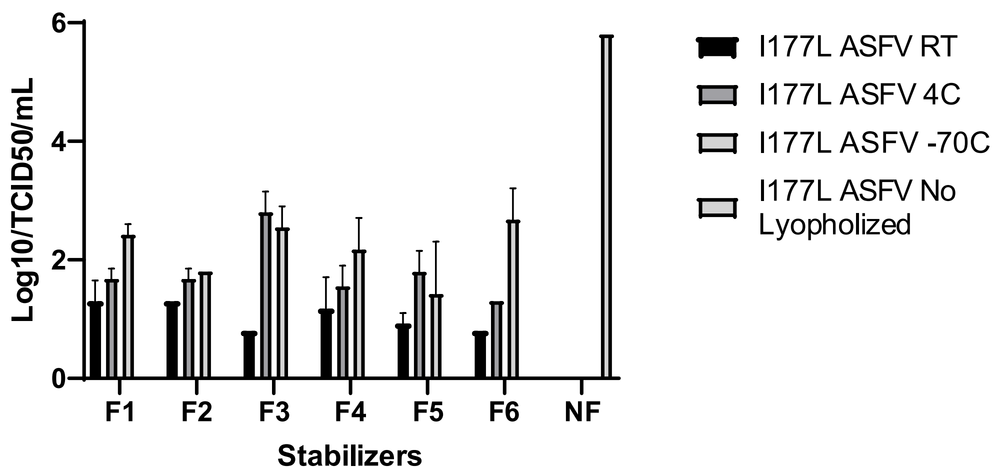

Figure 1 Screening results of stabilizer formulations. Six different stabilizer formulas were constructed. All formulas were lyophilized in the same batch. The samples were stored at three different temperatures (RT, 4C, and -70C). The results show the titer obtained after three weeks of lyophilization.

Batch two stablizers were modified to a 5:1 ratio rather than a 1:1 ration as in the first batch after three weeks, most of the dried products were collapsing. From this experiment, we decided to obtain one more screening step and test with the ratio/consistency of F2, F3, F5, and F6 to obtain a better product that will not collapse after a few days, batch two is based on the ratios of 5:1 for stablizer which were more stable on intial short term testing. In Batch two when stored at room temperature for up to 60 days, we observed that there were decreasing titers however some of the stabilizers F1, F4, F12, F13, appeared to be the most stable and were tested for further study (Figure 2).

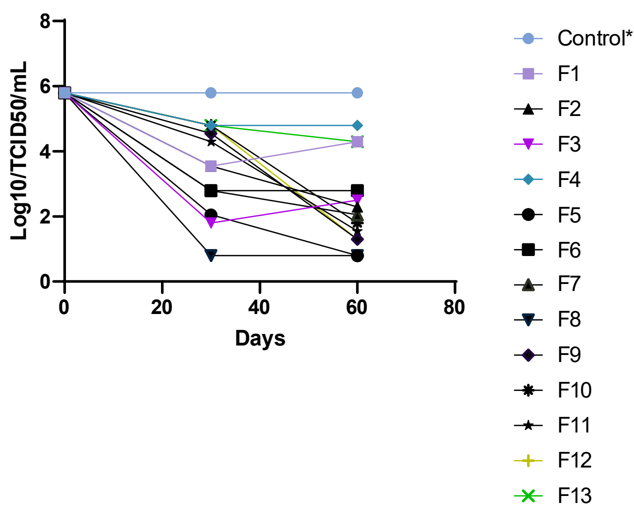

Figure 2. Screening of stabilizers stored at room temperature for a period of 60 days. The graph shows the titers obtained at 30 and 60 days after lyophilization of different formulations derived from the first screening.

In continued efforts we further optimized the lyophilization procedure. And we found that we could prevent some of the initial decrease in titer observed by using different drying procedures. (figure 3)

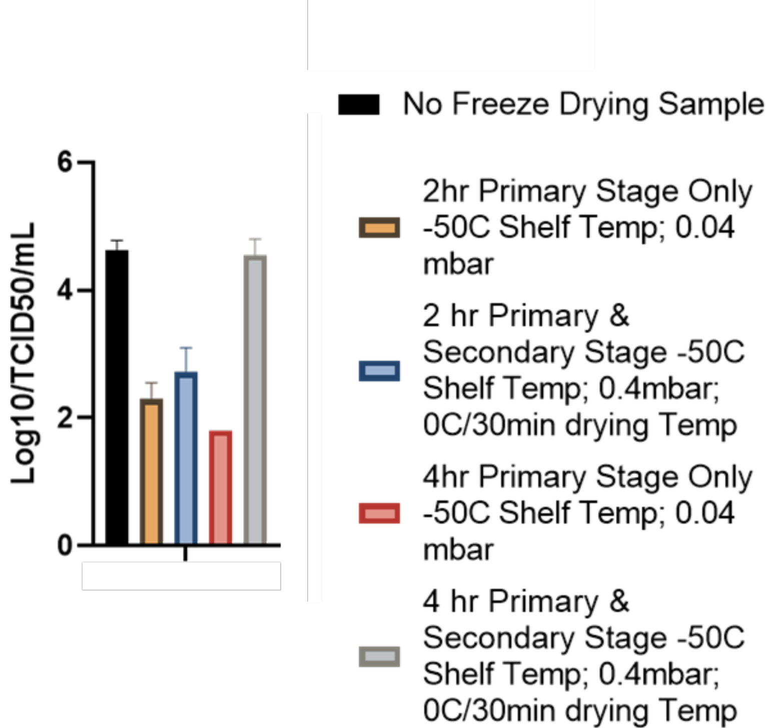

Figure 3: Fig. Effect of lyophilization on the survivability of ASFV-G-ΔI177L. Four different drying conditions were tested. The ideal sublimation phase was tested.

Using the increased lyophilization stage of 4 hours and an additional drying step than in the first two trials, resulted in increased stability. Trial 3 of freeze drying was conducted using this lyophilization step and using buffers F1, F3, F4, F5, F6, F12, F13 from trial 2.

Results from trial 3, show that there is a long term stability of at least 2 months at 4oC (figure 4)with different stabilization buffers and with at RT for Formulation 13 showing a slight decrease from earlier timepoints after two months.

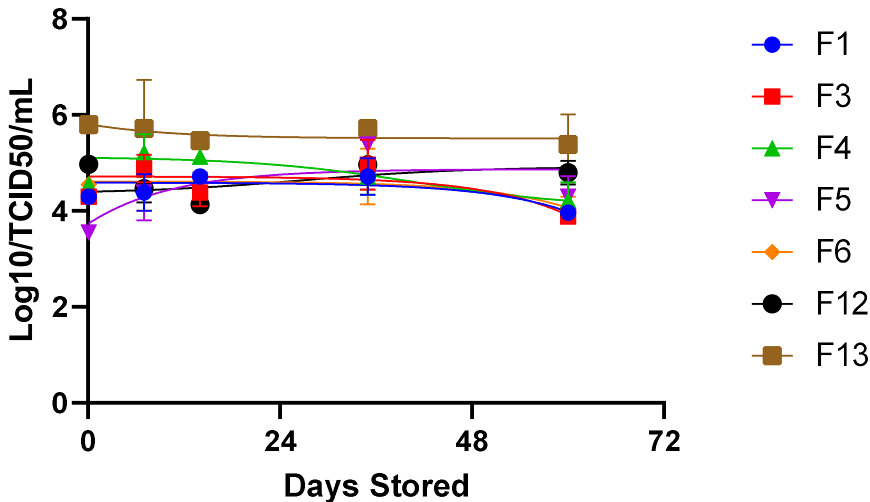

Figure 4: Screening of stabilizers stored at 4°C for a period of up to 60 days. The graph shows the titers obtained at 30 and 60 days after lyophilization of different formulations derived from the second batch, under new freeze drier settings.

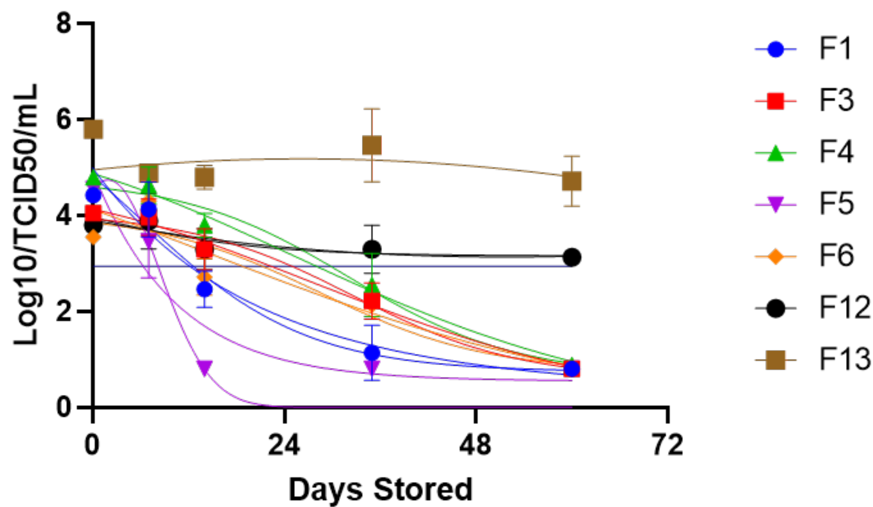

Figure 5: Screening of stabilizers stored at RT. for a period of up to 60 days. The graph shows the titers obtained at 30 and 60 days after lyophilization of different formulations derived from the second batch, under new freeze drier settings.

Objective 3: Test the ability of lyophilized ASFV to vaccinate swine against ASFV-Georgia.

The ability to test the lyophilized vaccine was proposed to be done after 6 months of lyophilized vaccine storage. Particularly if we continue to see a decrease in RT storage conditions. Funding for Objective 3 was from internal funds and will be performed when we reach 6 months of storage temperature, as the vaccine is able to infect cell culture, we do not expect to observe any differences in vaccine efficacy than that of the initial vaccine preparation that is stored at -70°C if the lyophilized vaccine remains stable at longer time periods.

Discussion: Lyophilization of ASFV vaccine candidates was expected to be straight forward with an expected slight decrease in titer when performed, however after our initial studies we realized that a stabilization buffer would be needed. After testing several stabilization buffers, we were able to test different conditions of freeze drying, including adding a drying step. Resulting in what appears to be a formulation F13 that appears to be extremely stable at 4°C, and at room temperature. This study will aid in the commercial distribution of any live attenuated vaccine for African swine fever, as it will not require the deep freezing of the initial vaccine formulation, we are continuing longer term studies to determine stability in longer time periods.