African Swine Fever Live Attenuated Virus Vaccine Safety, Efficacy and Evaluation in 3-week and 6-week-old Pigs

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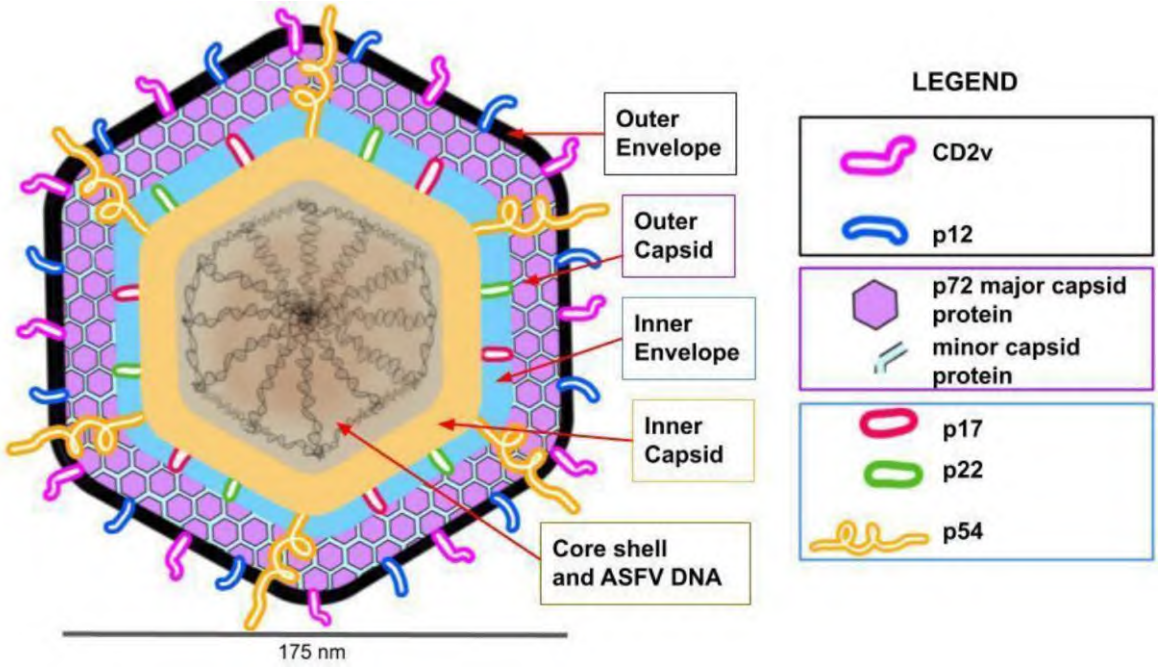
Outline

- African Swine Fever Virus (ASFV)
  - Status and Challenges
- ASFV LAV research development
  - 3-week old vs 6-week old pigs
- Research Directions

African Swine Fever Virus

- High consequence ASF etiological agent that causes high fever, loss of appetite, ataxia, depression and up to 100% mortality in pigs.
- First described in 1909 when it infected domestic pigs of European origin in Kenya. Confined in Africa in the long time.
- 1950s, 1960s outbreaks and eradication in 1990s by depopulation everywhere except in Africa.
- Notable outbreaks: 2007 Georgia, 2018 China.



Schematic illustration of ASFV virion
Buan et al., ImmunoInformatics. Vol 8, Dec 2022, 100019.

Why is it difficult to prevent and control ASF?

- the lack of a safe, efficient, commercial and readily available vaccines
- The need for a suitable cell line for wildtype ASFV isolation and propagation, and for large-scale LAV vaccine production.
- Availability of reliable (DIVA) diagnostic tests

ASFV is classified as Select Agent in the United States

- US HHS and USDA Select Agent List – biological agents and toxins determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products.
- ASFV has never been reported in the United States.
- ASFV classification
  - WHO Risk group 1 (RG1)
  - Working with ASFV requires BSL-3, ABSL-3 or BSL-3Ag containment facilities

Biosecurity Research Institute (BRI) at K-State

BRI

- BSL3 and BSL3Ag Research Facility at Manhattan, Kansas

NBAF

The BRI played a critical role in the selection of Manhattan as the site for the National Bio and Agro-Defense Facility.

BRI Personal Protective Equipment - ASFV

BRI Large Animal Room(s)

- Scrubs and socks
- Rubber boots
- Tyvek
- Double gloves
- Eye & ear protection
- N95 Respirator

ASF Laboratory

- Scrubs and socks
- Crocs
- Disposable lab gown
- Double gloves
- Eye protection

- Shower out required when exiting lab and animal rooms
- 5-day quarantine from pigs

ASF LAVs generated by serial passages of ASFV field isolates (genotype II)

Dr. Quang Lam Truong' group, Vietnam National University of Agriculture, Vietnam.

VNUA-ASFV-LAVL2: KNB-LAV1

VNUA-ASFV-LAVL3: KNB-LAV2

› Viruses. 2023 Oct 13;15(10):2089. doi: 10.3390/v15102089.

A Cell-Adapted Live-Attenuated Vaccine Candidate Protects Pigs against the Homologous Strain VNUA-ASFV-05L1, a Representative Strain of the Contemporary Pandemic African Swine Fever Virus

Quang Lam Truong [1], Lihua Wang [2], Tuan Anh Nguyen [1], Hoa Thi Nguyen [1], Son Danh Tran [1], Anh Thi Vu [1], Anh Dao Le [1], Van Giap Nguyen [3], Phuong Thi Hoang [1], Yen Thi Nguyen [1], Thi Luyen Le [1], Thang Nguyen Van [1], Thi My Le Huynh [3], Huong Thi Lan Lai [1], Rachel Madera [2], Yuzhen Li [2], Jishu Shi [2], Lan Thi Nguyen [1]

Affiliations – collapse

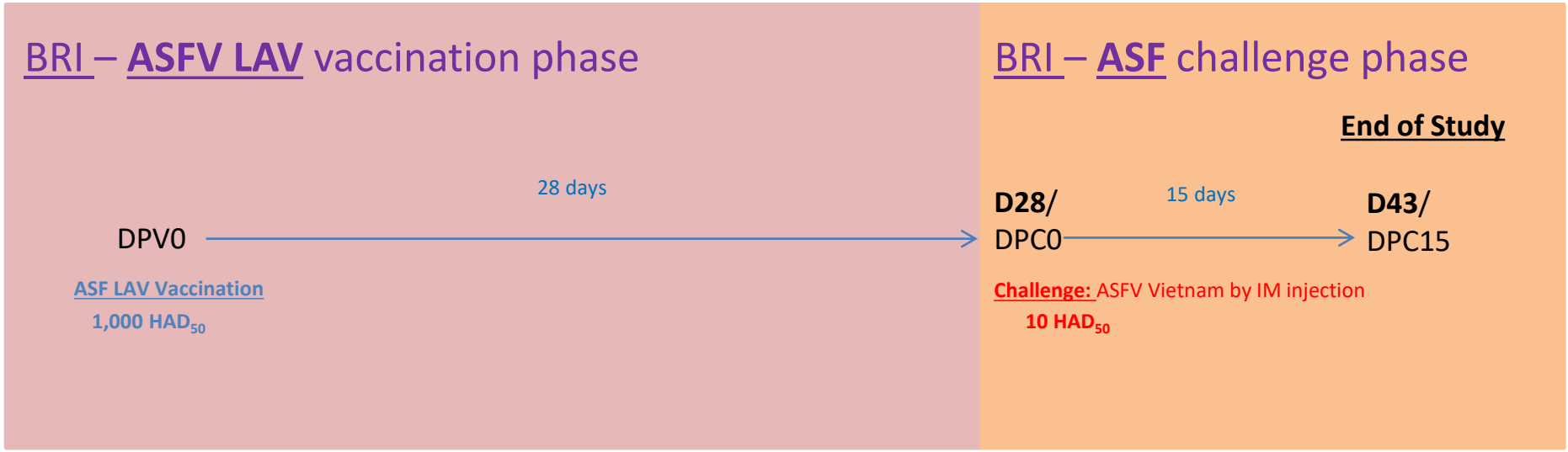
Affiliations

1 Key Laboratory of Veterinary Biotechnology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Gia Lam, Ha Noi 12406, Vietnam.
2 Center on Vaccine Evaluation and Alternatives for Antimicrobials, Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA.
3 Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Gia Lam, Ha Noi 12406, Vietnam.

PMID: 37896866 PMCID: PMC10612049 DOI: 10.3390/v15102089

KNB-LAV1 evaluation by vaccination and challenge studies in pigs

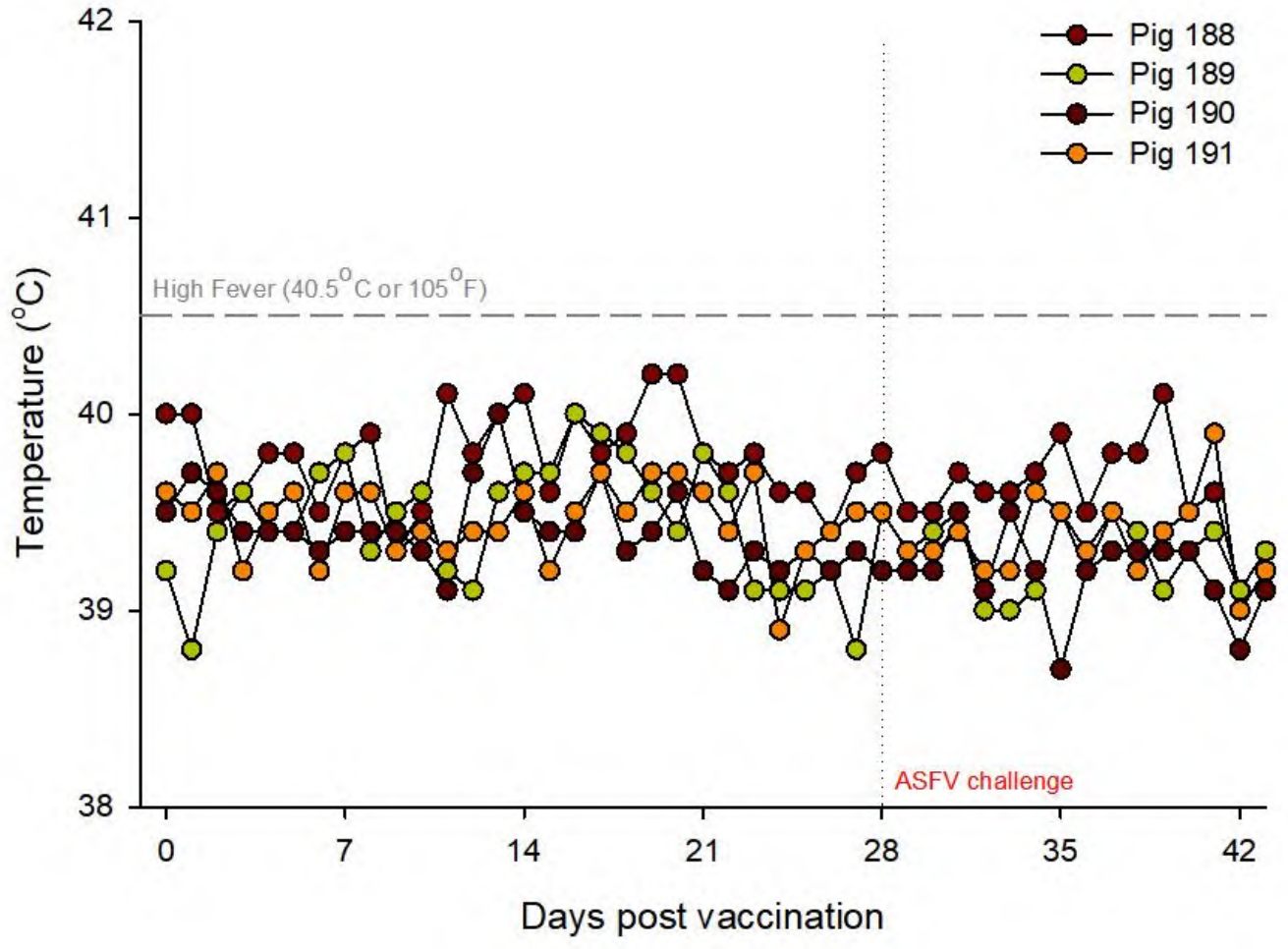
Study Timeline **3-wk old pigs**



- Blood and Weight Collection
- Daily Temperature Monitoring using thermal microchips

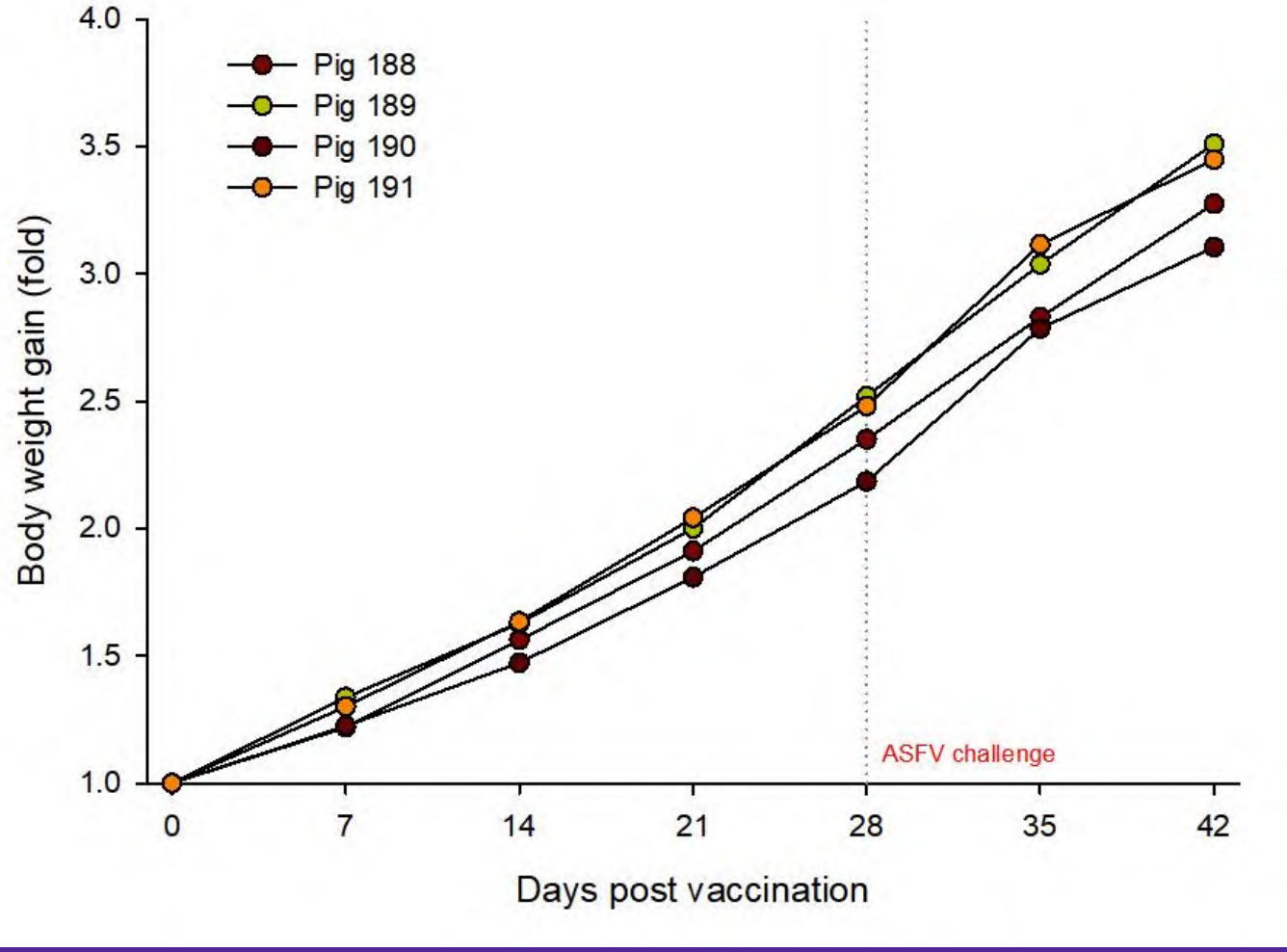
KNB-LAV1 safety and efficacy evaluation in pigs

- KNB-LAV1 vaccinated pigs did not exhibit high temperatures during vaccination and challenge phases



KNB-LAV1 safety and efficacy evaluation in pigs

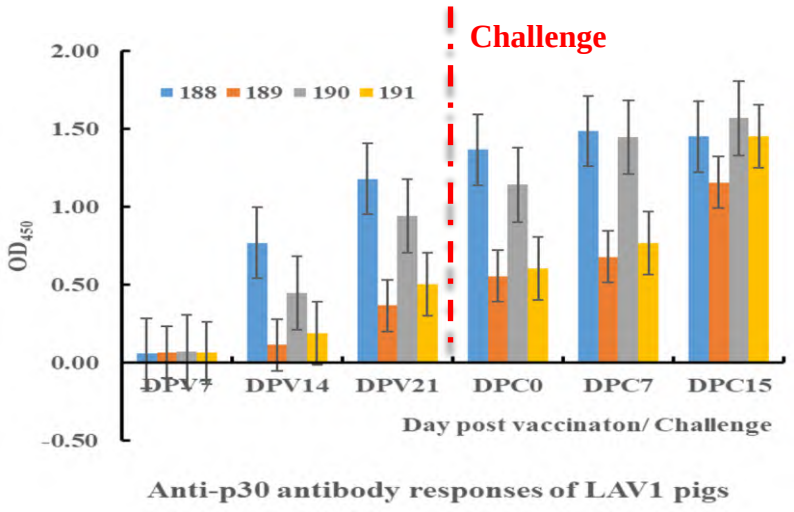
- KNB-LAV1 vaccinated pigs continued to gain weight during vaccination and challenge phases



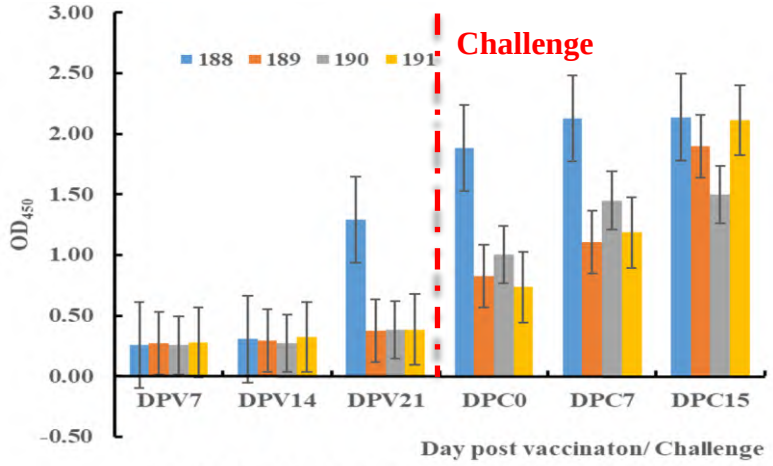
KNB-LAV1 safety and efficacy evaluation in pigs

- KNB-LAV1 vaccinated pigs exhibited ASFV-specific antibody responses

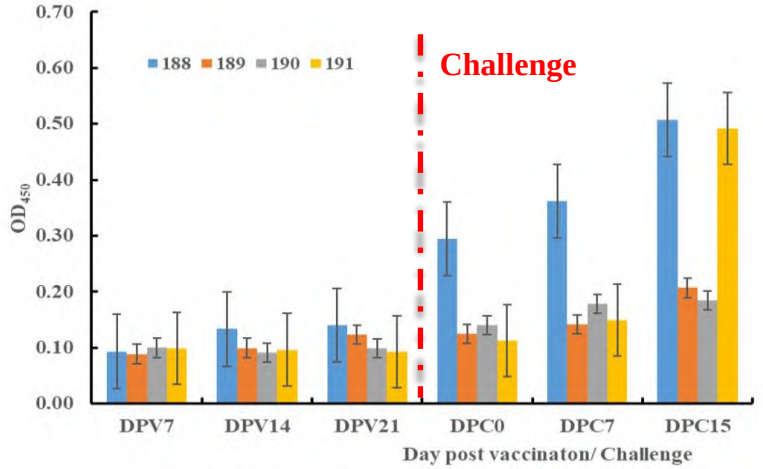
inner envelop membrane proteins



Anti-p30 antibody responses of LAV1 pigs



Anti-p17 antibody responses of LAV1 pigs



Anti-p54 antibody responses of LAV1 pigs

KNB-LAV1 safety and efficacy evaluation in pigs

- KNB-LAV1 vaccinated pigs exhibited little or no viremia during vaccination and challenge phases

	DPV0		DPV7		DPV14		DPV21		DPV28/DPC0		DPC7		DPC14		DPC15	
Tag#	Ct	Quantity	Ct	Quantity	Ct	Quantity	Ct	Quantity	Ct	Quantity	Ct	Quantity	Ct	Quantity	Ct	Quantity
188	Undetermined		Undetermined		Undetermined		40.75	0.25	Undetermined		Undetermined		Undetermined		Undetermined	
189	Undetermined		Undetermined		Undetermined		35.57	10.02	Undetermined		Undetermined		Undetermined		Undetermined	
190	Undetermined		Undetermined		36.50	5.16	Undetermined		Undetermined		Undetermined		Undetermined		Undetermined	
191	Undetermined		Undetermined		Undetermined		Undetermined		Undetermined		Undetermined		Undetermined		36.06	36.01
192	Undetermined		Undetermined		Undetermined		Undetermined		Undetermined		21.13	299,454.53				
193	Undetermined		Undetermined		Undetermined		37.21	3.12								

- Little or no viremia detected in sentinel pigs during vaccination phase.
  - Pig 193 (DPV26) potentially non-ASF related (not tested). Pig 192 (DPC9).

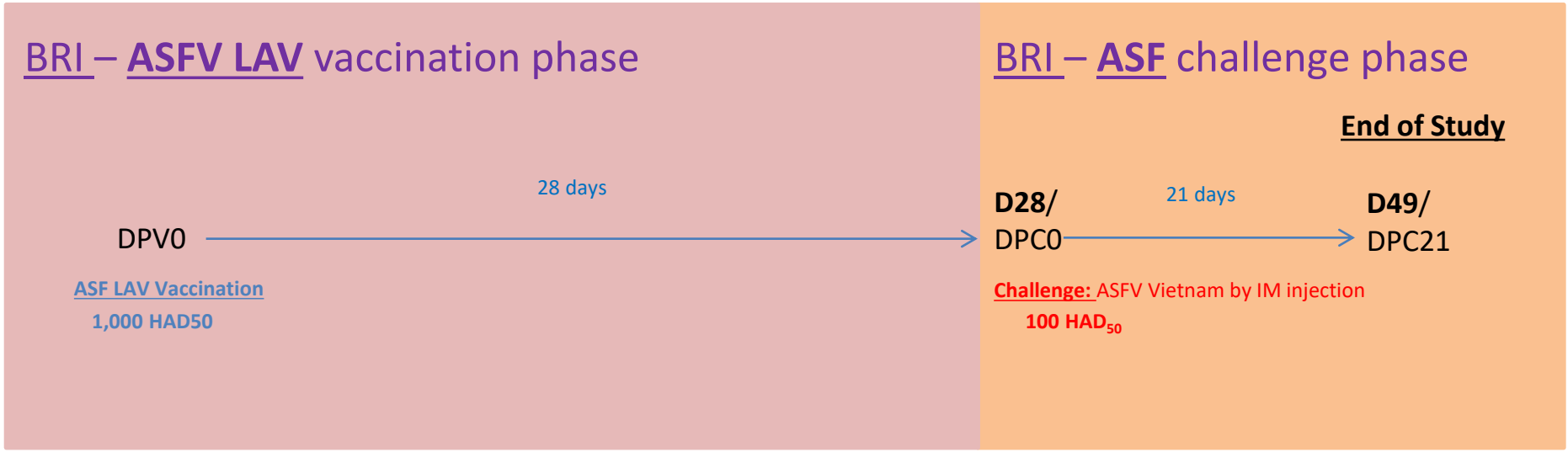
KNB-LAV1 safety and efficacy evaluation in pigs

- KNB-LAV1 vaccinated pigs did not exhibit high temperatures during vaccination and challenge phases
  - continued to gain weight
  - exhibited ASFV-specific antibody responses
  - exhibited little or no viremia during vaccination and challenge phases

KNB-LAV2 safety and efficacy evaluation in pigs

Study Timeline

3-wk old pigs and 6-wk old pigs



- Blood and Weight Collection
- Daily Temperature Monitoring using thermal microchips

KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

3-wk old pigs

- 6 vaccinated pigs
- 2 sentinels
- 4 (-/+) control pigs

- 4 (-/-) control pigs

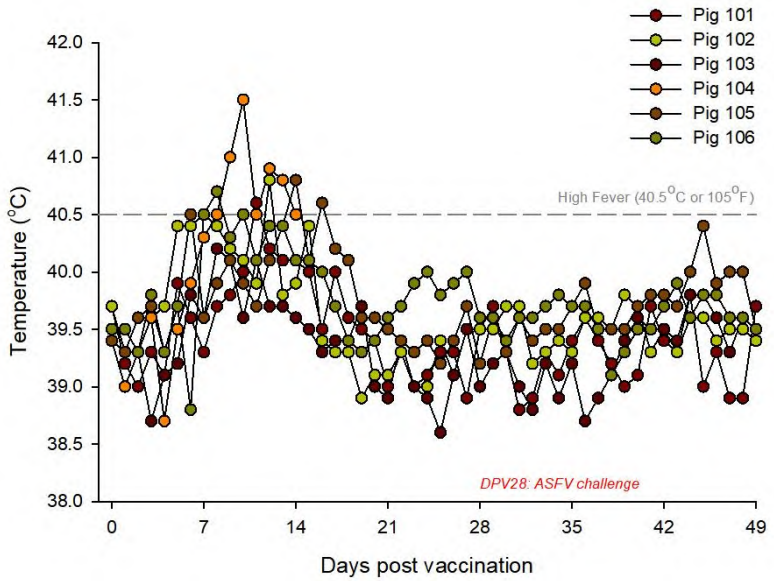
6-wk old pigs

- 6 vaccinated pigs
- 2 sentinels
- 4 (-/+) control pigs

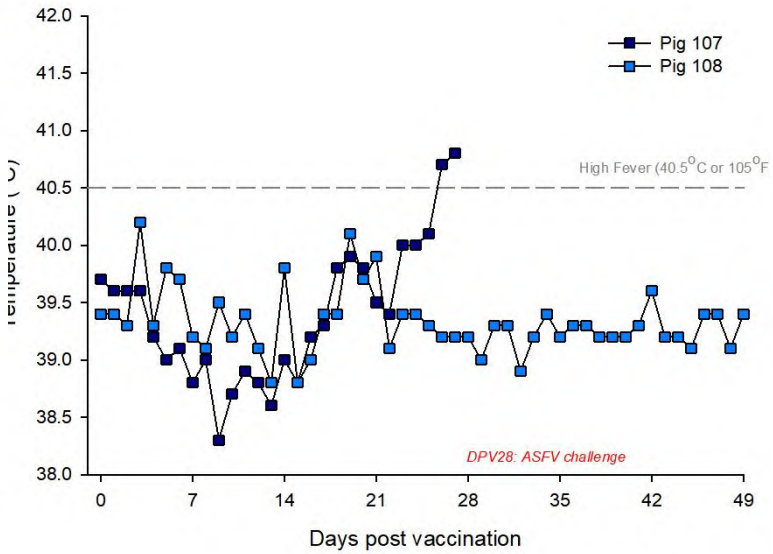
KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

3-wk old pigs - Temperature

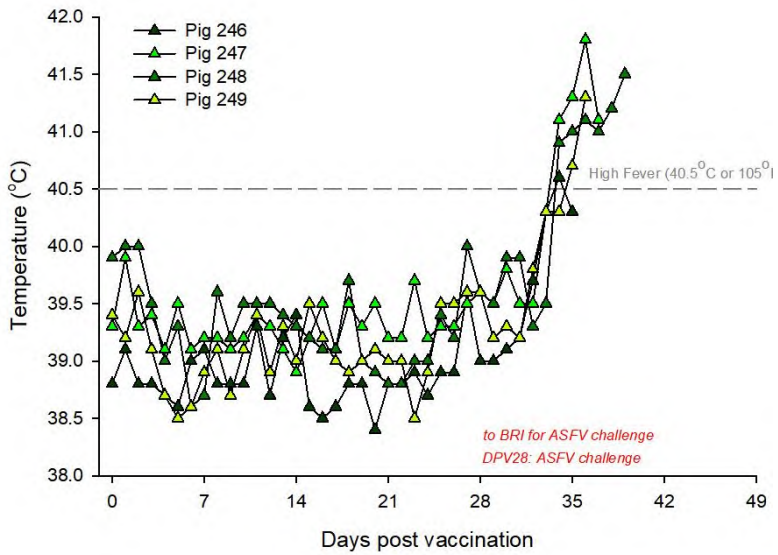
LAV vaccinated pigs



Sentinel pigs



(-/+) control pigs

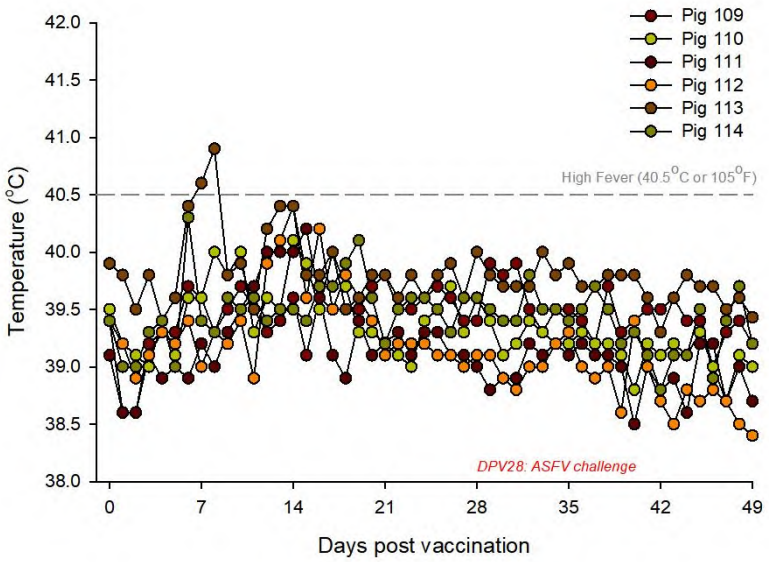


- All 3-wk LAV vaccinated pigs survived ASFV challenge. Pig 104 euthanized at DPV14.
- Sentinel pig 107 was euthanized before challenge. Sentinel pig 108 survived ASFV challenge
- All (-/+) pigs did not survive ASFV challenge

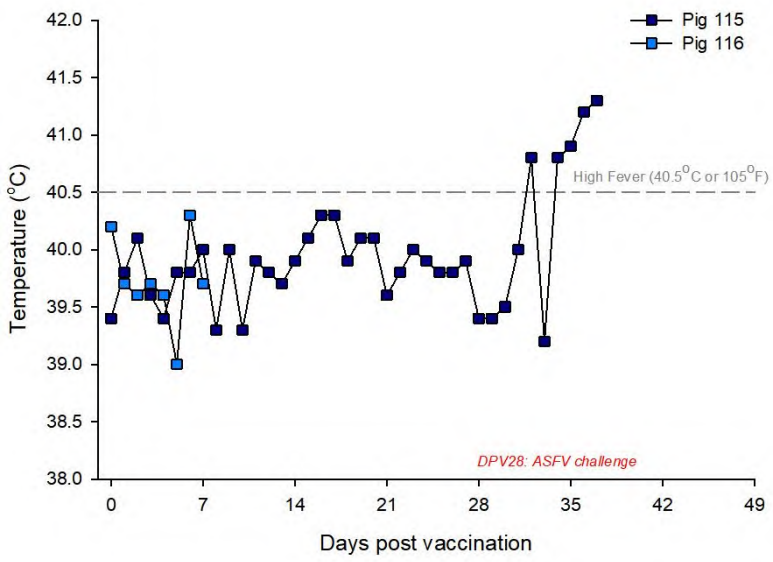
KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

6-wk old pigs - Temperature

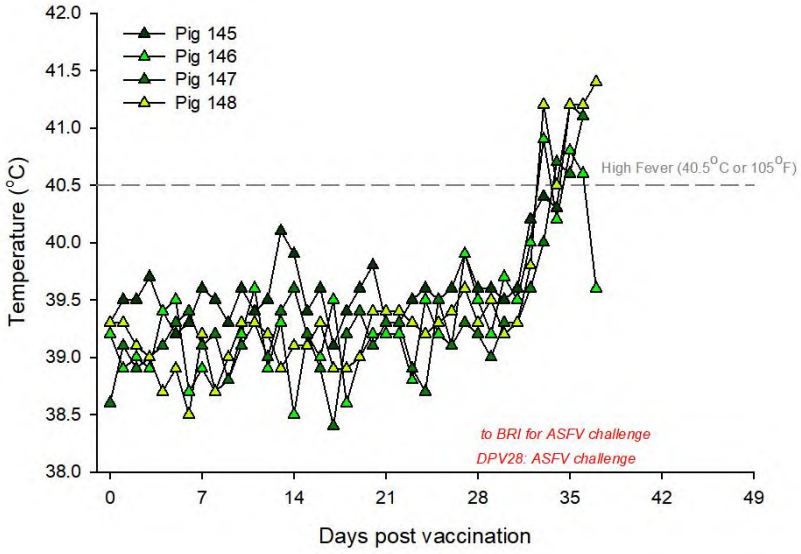
LAV vaccinated pigs



Sentinel pigs



(-/+) control pigs

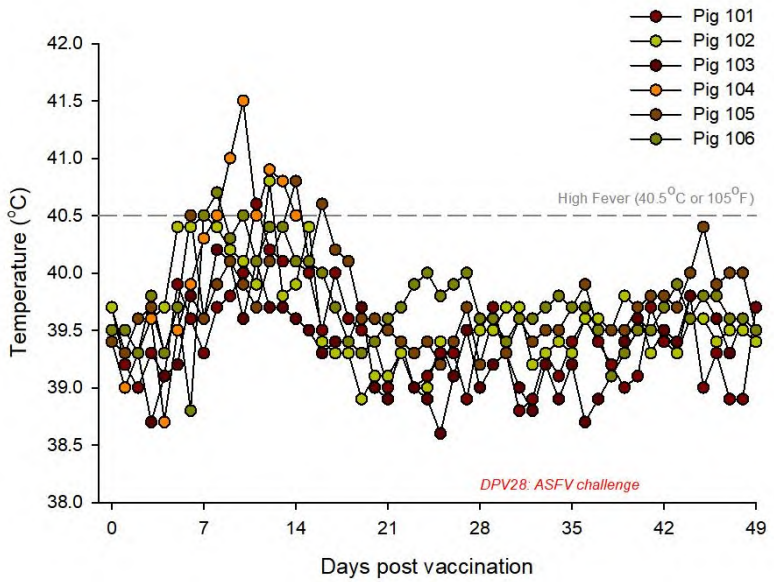


- All 6-wk LAV vaccinated pigs survived ASFV challenge.
- Sentinel pig 116 was euthanized before challenge (non-ASF). Sentinel pig 115 did not survive ASFV challenge
- All (-/+) pigs did not survive ASFV challenge

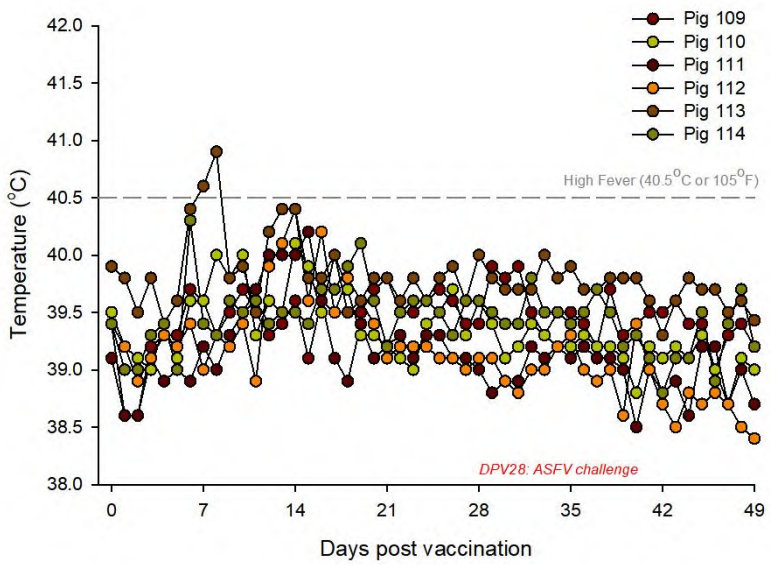
KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

Temperature: 3-wk old pigs vs 6-wk old pigs vs (-/-) control pigs

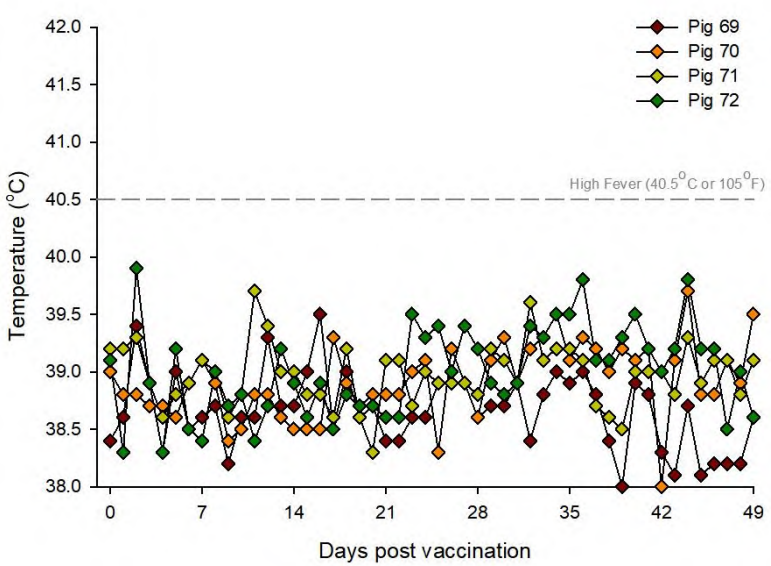
3-wk LAV pigs



6-wk LAV pigs



(-/-) control pigs

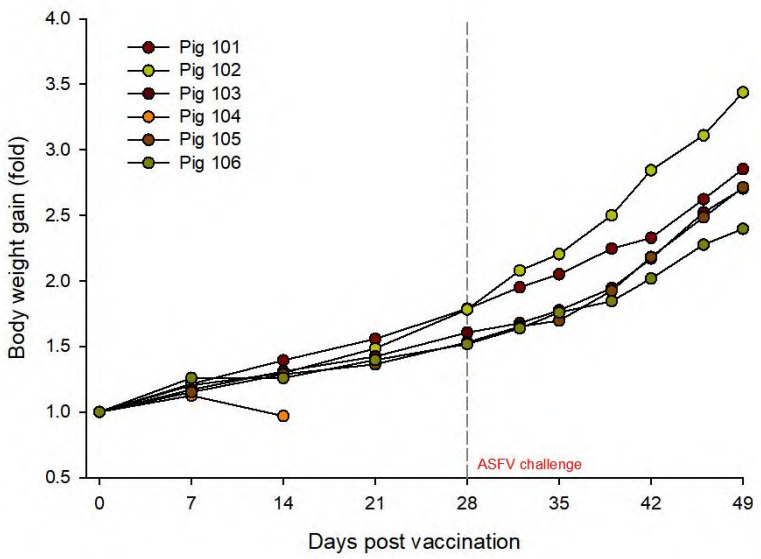


- Slight increase in temperatures observed at 1 to 2 weeks post vaccination in LAV pigs.

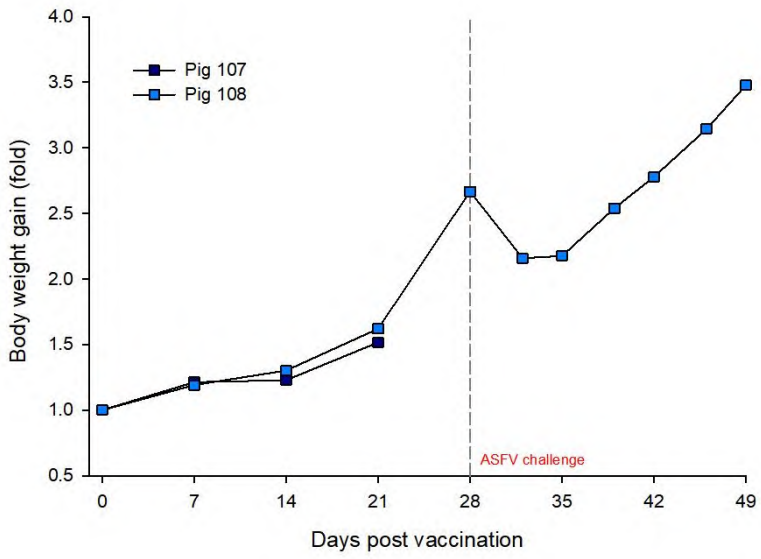
KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

3-wk old pigs – weight gain

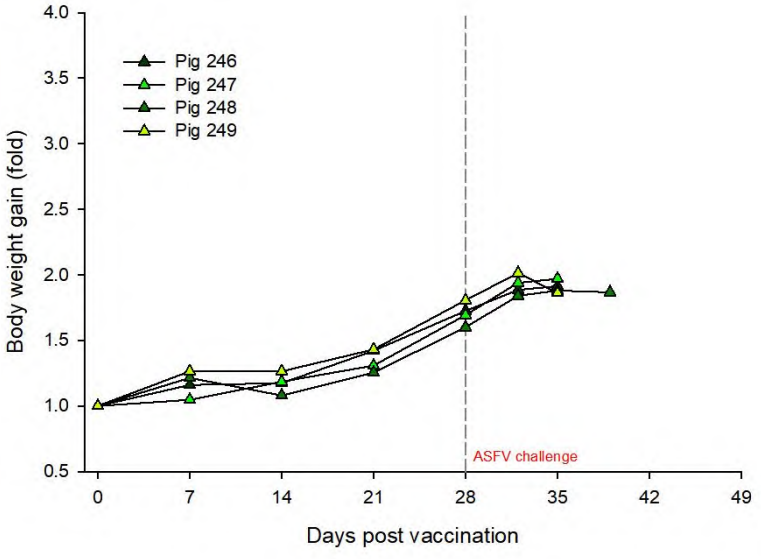
LAV vaccinated pigs



Sentinel pigs



(-/+) control pigs

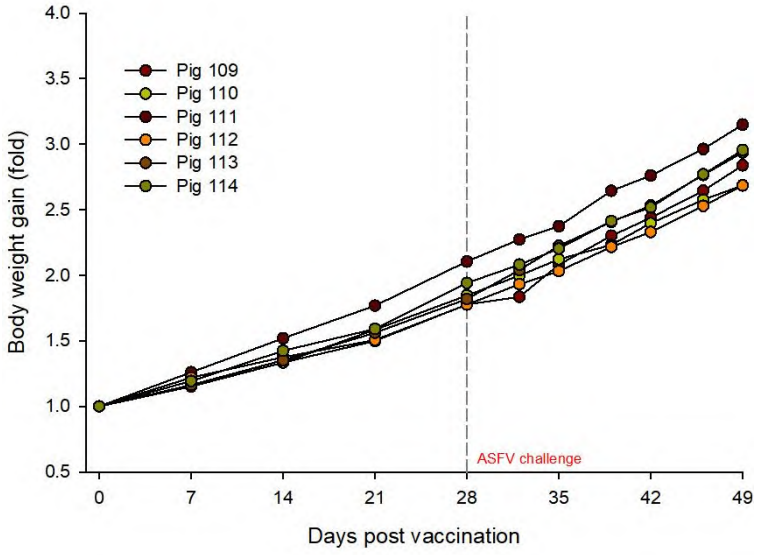


- All 3-wk LAV vaccinated pigs continued to gain weight even during ASFV challenge phase
- Sentinel pig 107 was euthanized before challenge. Sentinel pig 108 lost weight after challenge and eventually continued to gain weight; survived ASFV challenge.
- All (-/+) pigs did not gain weight and died during ASFV challenge phase.

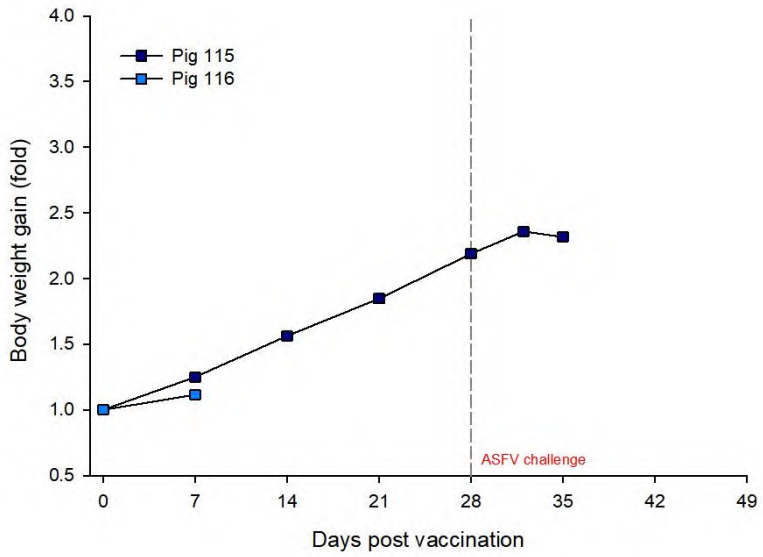
KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

6-wk old pigs – weight gain

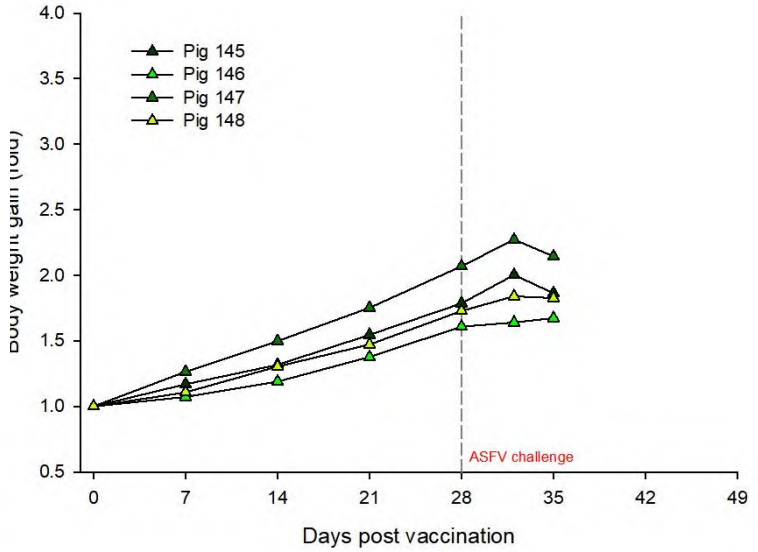
LAV vaccinated pigs



Sentinel pigs



(-/+) control pigs

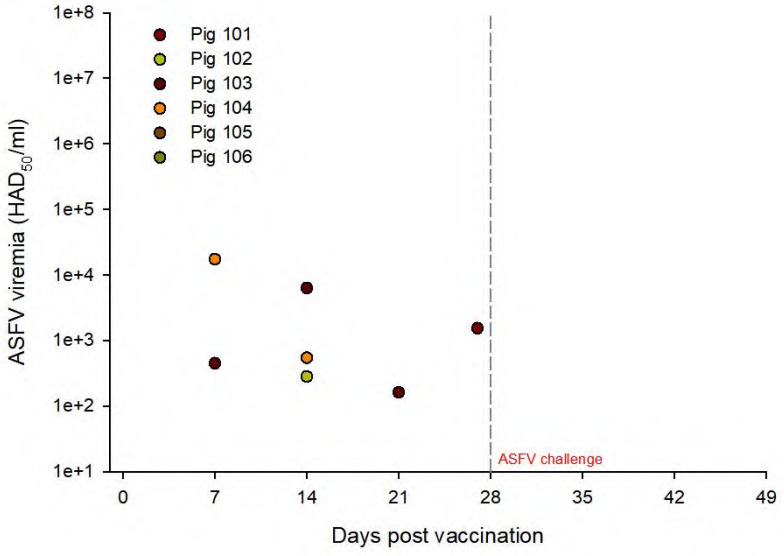


- All 6-wk LAV vaccinated pigs continued to gain weight even during ASFV challenge phase
- Sentinel pig 116 was euthanized before challenge (non-ASF). Sentinel pig 115 did not survive ASFV challenge
- All (-/+) pigs lost weight and died during ASFV challenge phase.

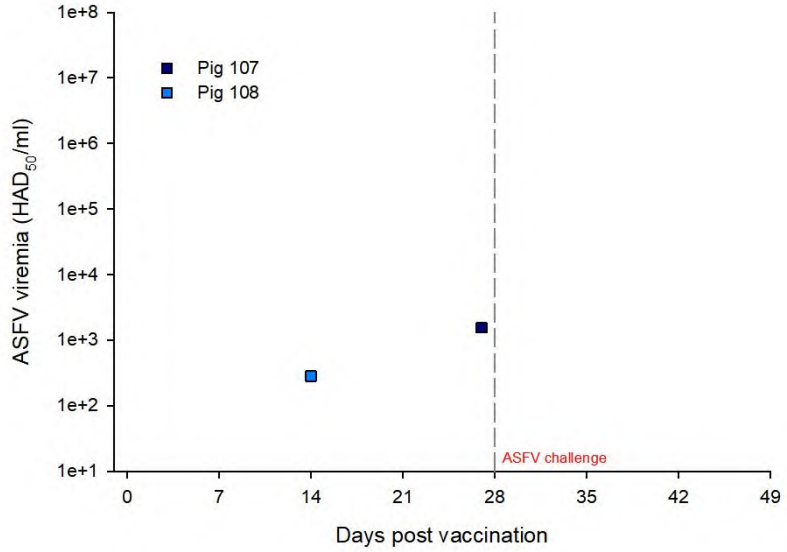
KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

3-wk old pigs – ASF viremia by RT-PCR

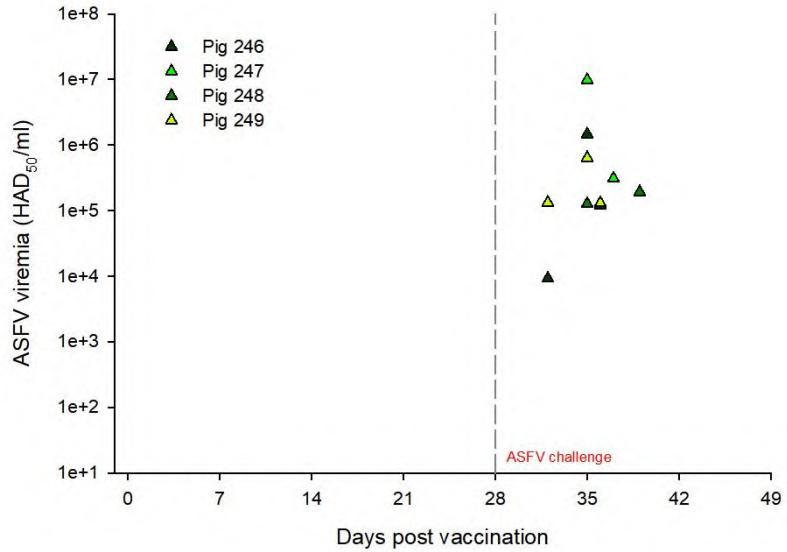
LAV vaccinated pigs



Sentinel pigs



(-/+) control pigs

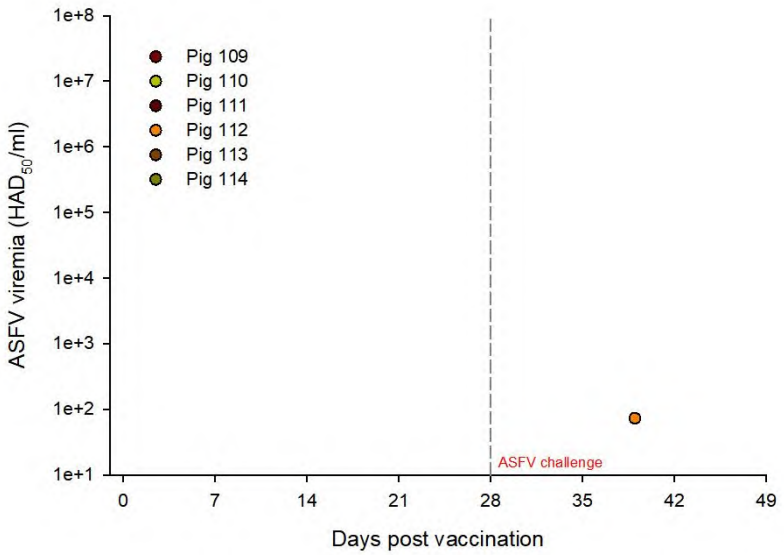


- 3-wk LAV vaccinated and 3-wk sentinel pigs tested positive for ASFV during vaccination phase – indicative of LAV shedding.
- Sentinel pig 108 turned viremic at DPV14 *but survived challenge*. Sentinel pig 107 exhibited low viremia towards end of vaccination phase, evaluated not fit for ASF challenge, euthanized.
- (-/+) pigs were euthanized between DPV36/DPC8 to DPV39/DPC11.

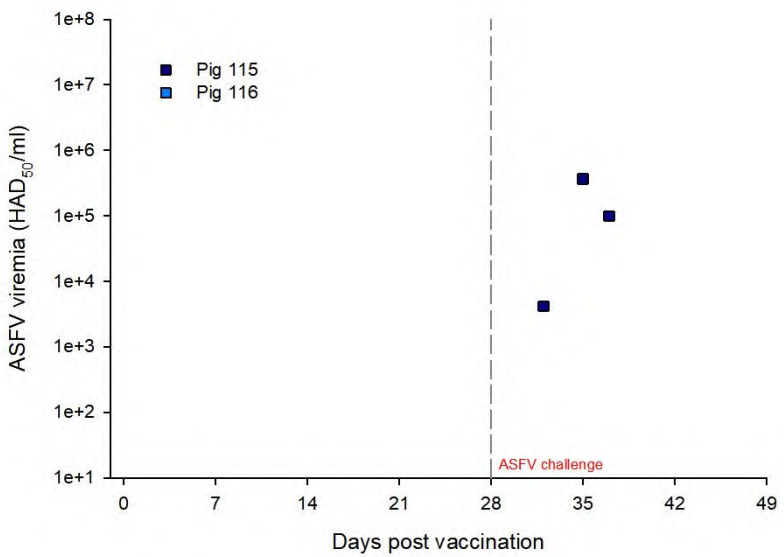
KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

6-wk old pigs – ASF viremia by RT-PCR

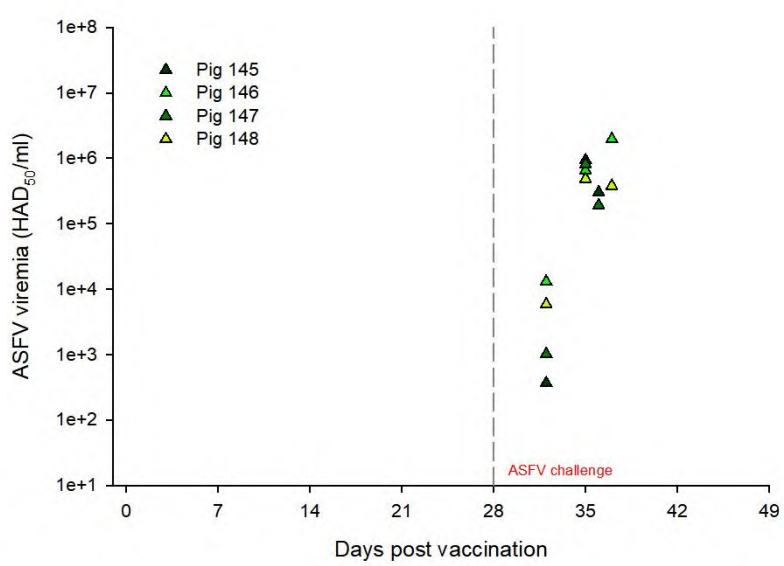
LAV vaccinated pigs



Sentinel pigs



(-/+) control pigs

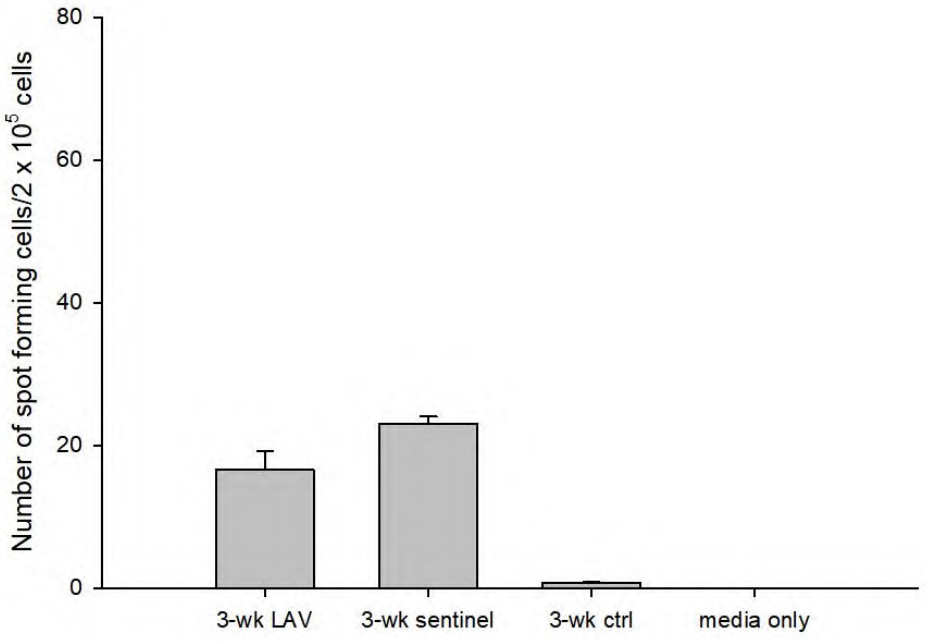


- 6-wk LAV vaccinated and 6-wk sentinel pigs tested negative for ASFV during vaccination phase.
- Sentinel pig 116 (DPV7) was non-viremic. Pig 115 also non-viremic during vaccination phase and did not survive ASF challenge (DPC9).
- (-/+) pigs were euthanized between DPV36/DPC8 to DPV37/DPC9.

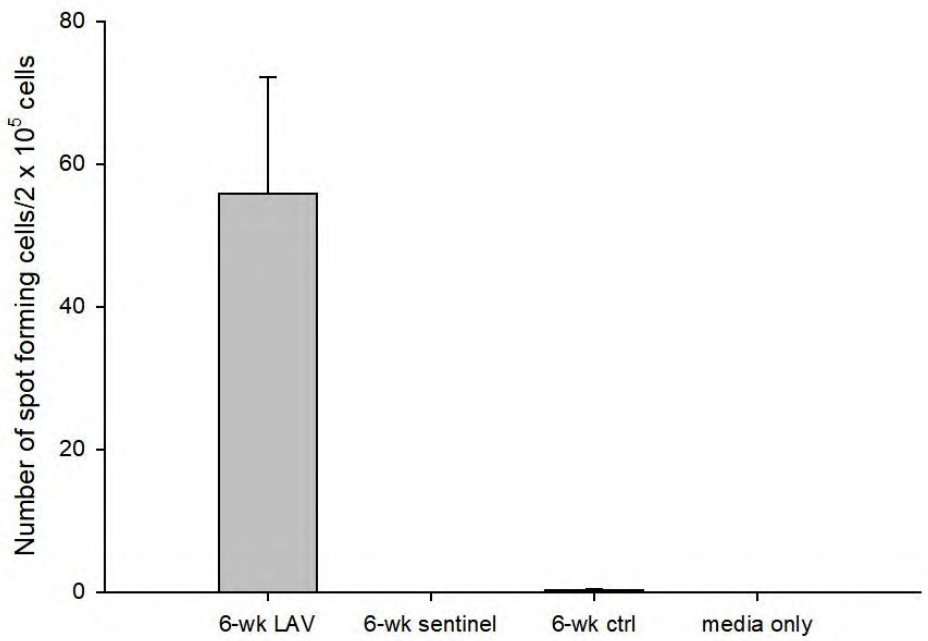
KNB-LAV2 evaluation in 3-wk old and 6-wk old pigs

Cell-mediated Responses at DPV28/DPC0: ELISPOT Analysis

3-wk LAV pigs



6-wk LAV pigs



- 3-wk sentinel pigs appear to have acquired ASFV-specific cell mediated responses. No ASFV-specific cell mediated responses observed in 6-wk sentinel pig..
- Higher IFN-gamma producing cells observed in 6-wk old pigs.

Survival of ASFV-challenged pigs

	3-wk old pigs	6-wk old pigs
LAV vaccinated pigs	5/5 or 100%	6/6 or 100%
Sentinels	1/1 or 100%	0/1 or 0%
(-/+) control pigs	0/4 or 0%	0/4 or 0%

- 3-wk old sentinel surviving ASFV challenge potentially indicate that shedding occurred during vaccination phase

Summary

- Controlled/non-field study on ASF LAV vaccination of 3-wk and 6-wk old pigs.
  - vaccinated pigs survived ASFV challenge
  - Horizontal transmission observed in 3-wk vaccinated pigs, none in 6-wk pigs
- Future directions
  - Conduct further vaccination and challenge studies
  - Conduct further attenuation by serial passaging of the virus for safer and more effective ASF LAV.

Acknowledgements

Center on Vaccine Evaluation and Alternatives for Antimicrobials, Department of Anatomy & Physiology College of Veterinary Medicine, KSU

Biosecurity Research Institute (BRI) and Comparative Medicine Group at KSU

Dr. Quang Lam Truong at VNUA

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