

# Genomic Surveillance Reveals High Genotypic Diversity Among Genotype I African Swine Fever Virus Strains in Zambia

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GLOBAL AFRICAN SWINE FEVER RESEARCH ALLIANCE

KAMPALA, UGANDA

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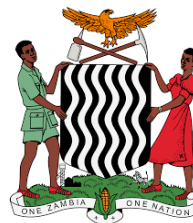
## **GARA**

### **GAP ANALYSIS WORKSHOP**

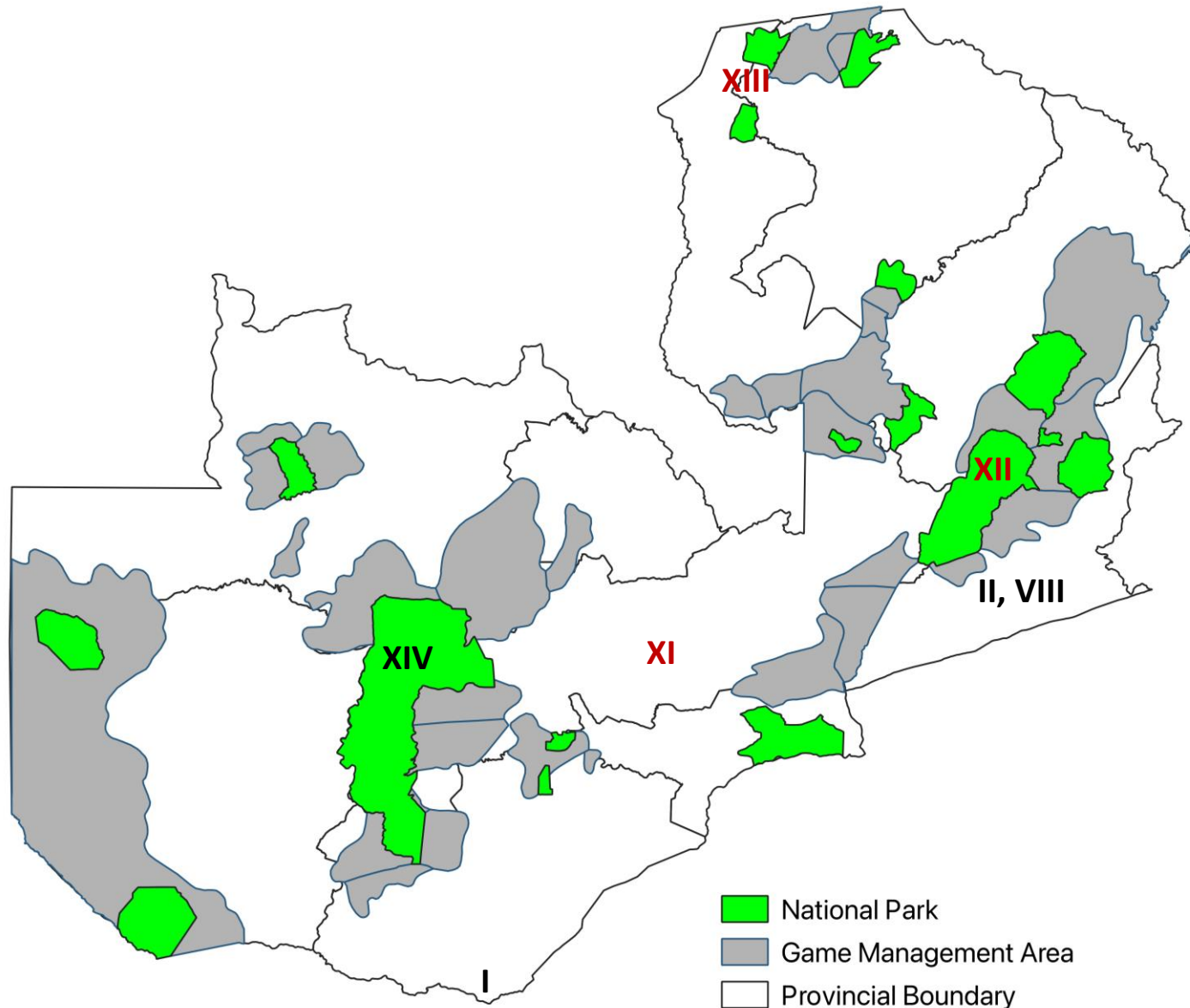
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# Epidemiology of ASF in Zambia



- **7 genotypes identified so far**
  - ❖ I, II, VIII, XIV (Outbreaks)
  - ❖ XI, XII, & XIII (Ticks)
- **Previous studies**
  - ✓ Primarily relied on disease outbreaks (*Simulundu et al., 2017, Viruses*)
- **However, there is no data on;**
  - ✓ Circulating ASFV serotypes
  - ✓ Genetic diversity of tick ASFVs
- **Moreover**
  - ✓ ASFV surveillance in soft ticks was conducted over 30 years ago

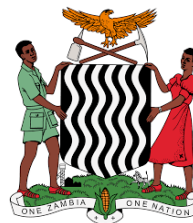
# Aim & Objectives of the Study

## ○ AIM

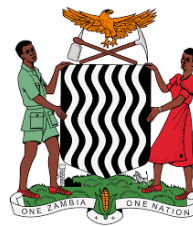
- ✓ To understand epidemiology of ASF in Zambia

## ○ Objectives

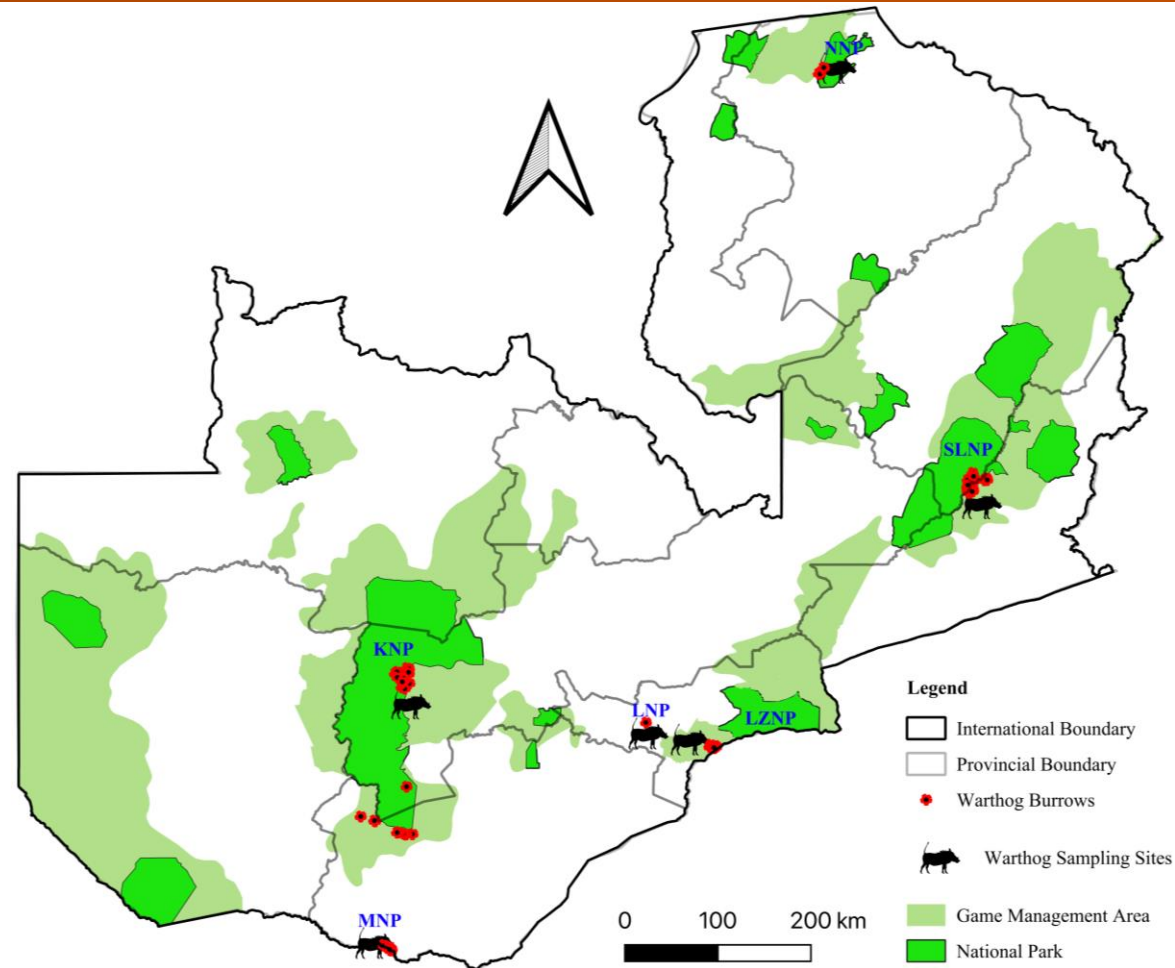
1. To conduct surveillance for ASF at the wildlife-livestock interface area
2. To determine the genetic diversity & circulating serotypes of ASFVs in Zambia




# Methods & Results



# Sample Collection & Processing

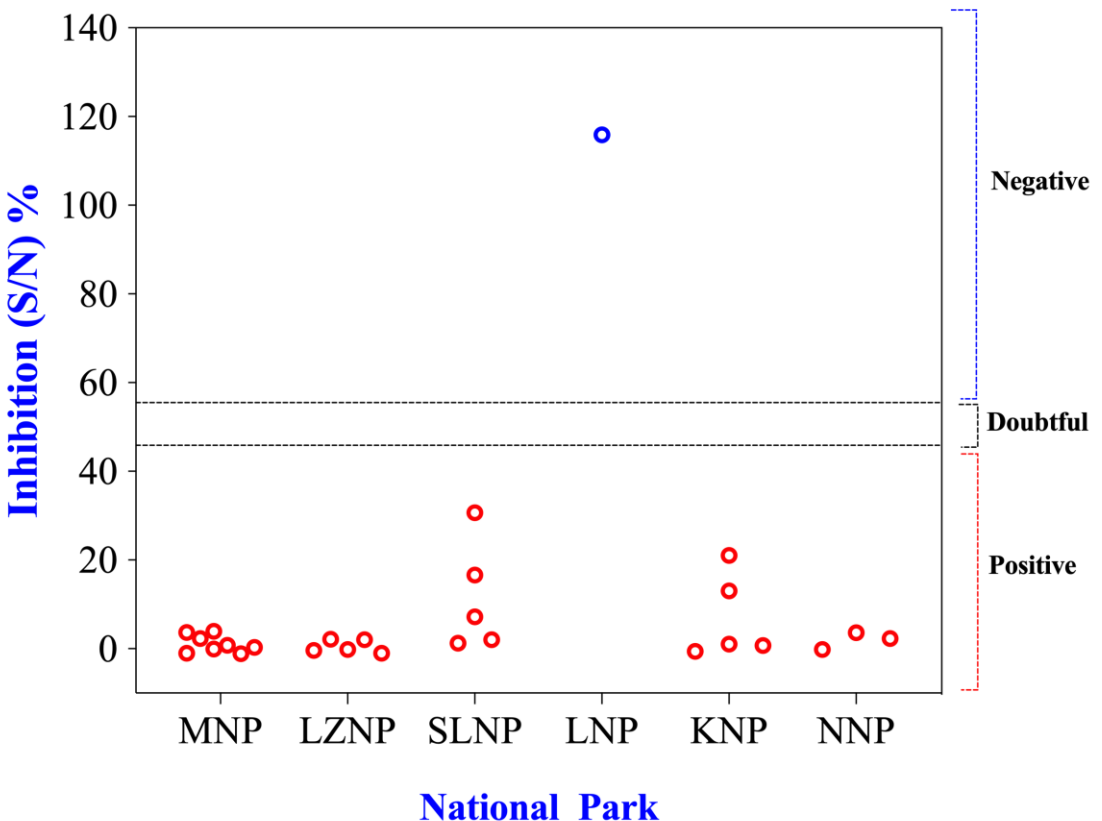


- **Warthogs [Blood & Tissues]**
  - ✓ KNP: 5; SLNP: 5; MNP: 3; LZNP: 5; NNP: 3
- **Soft Ticks: [ $n=9,717$ ]**
  - ✓  KNP, SLNP, & MNP: 300 pools
  - ✓ LZNP: 46 & NNP: 25 [*Under analysis*]
  - ❖ DNA extraction, PCR & sequencing
- **Additionally, ASFV outbreak strains** (*Simulundu et al., 2017, Viruses*)
  - ✓ Genotype I ( $n=4$ ); II ( $n=1$ ) & XIV ( $n=1$ )
  - ❖ Were included for serotyping
- **ASFV genome detection in ticks**
  - ❖ OIE qPCR assay (*king et al., 2003, J Virol Methods*)
- **ASFV serotyping (CD2v genotyping)**
  - ❖ In-house PCR assay (*unpublished*)
- **ASFV Antibody detection in warthogs**
  - ❖ ELISA: ID Screen competition ELISA

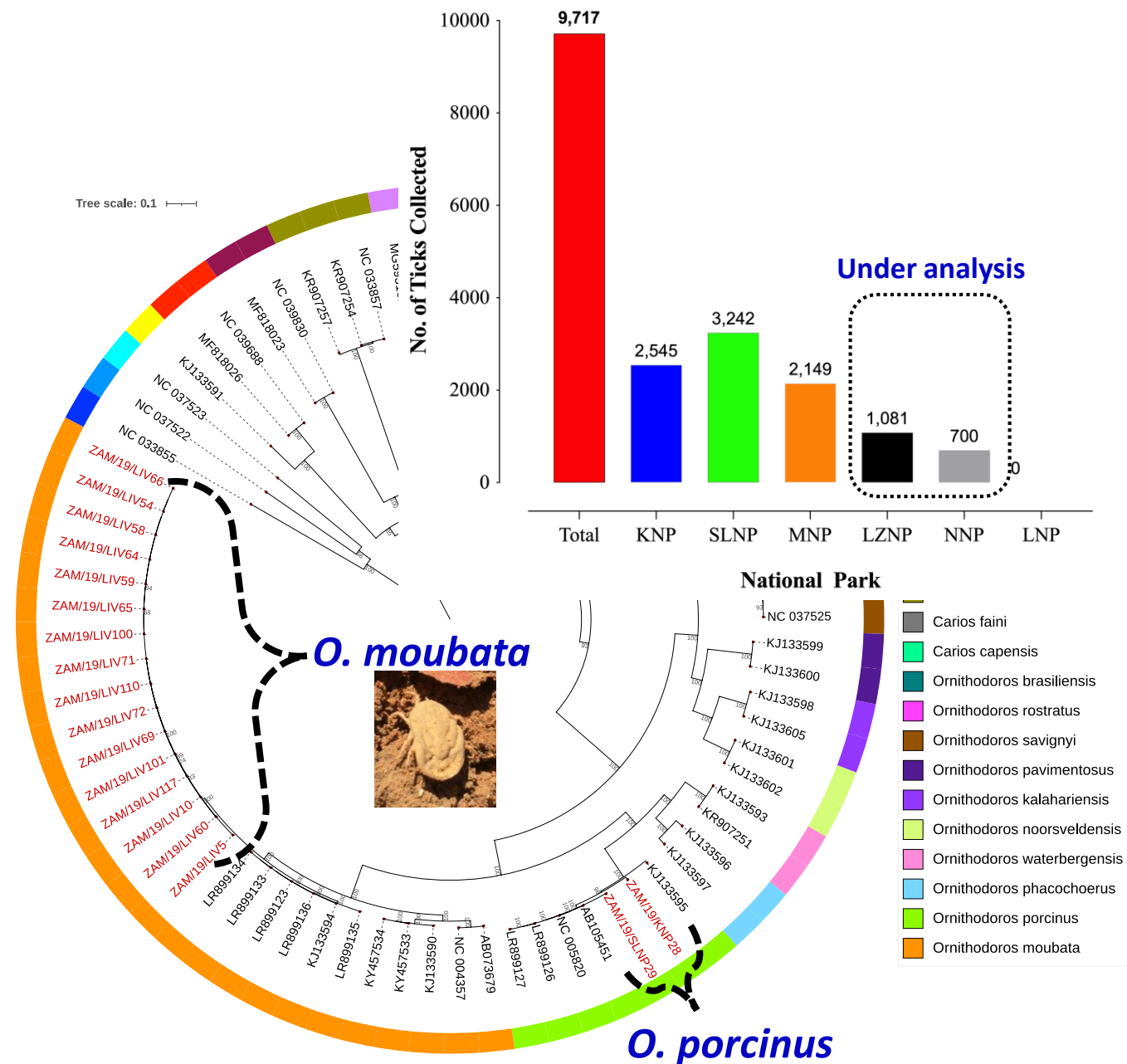


# ASFV Antibody Detection & Soft Tick Identification

## ASFV Antibody Detection in Warthogs



- ✓ High seroprevalence of ASFV antibodies in warthogs
- ❖ ASFV genome detection is ongoing





# Genome Detection & Genotyping of Tick ASFVs

## ○ In This study;

✓ 3 Genotypes were identified from soft ticks

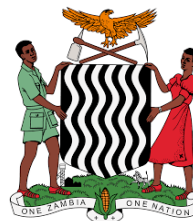
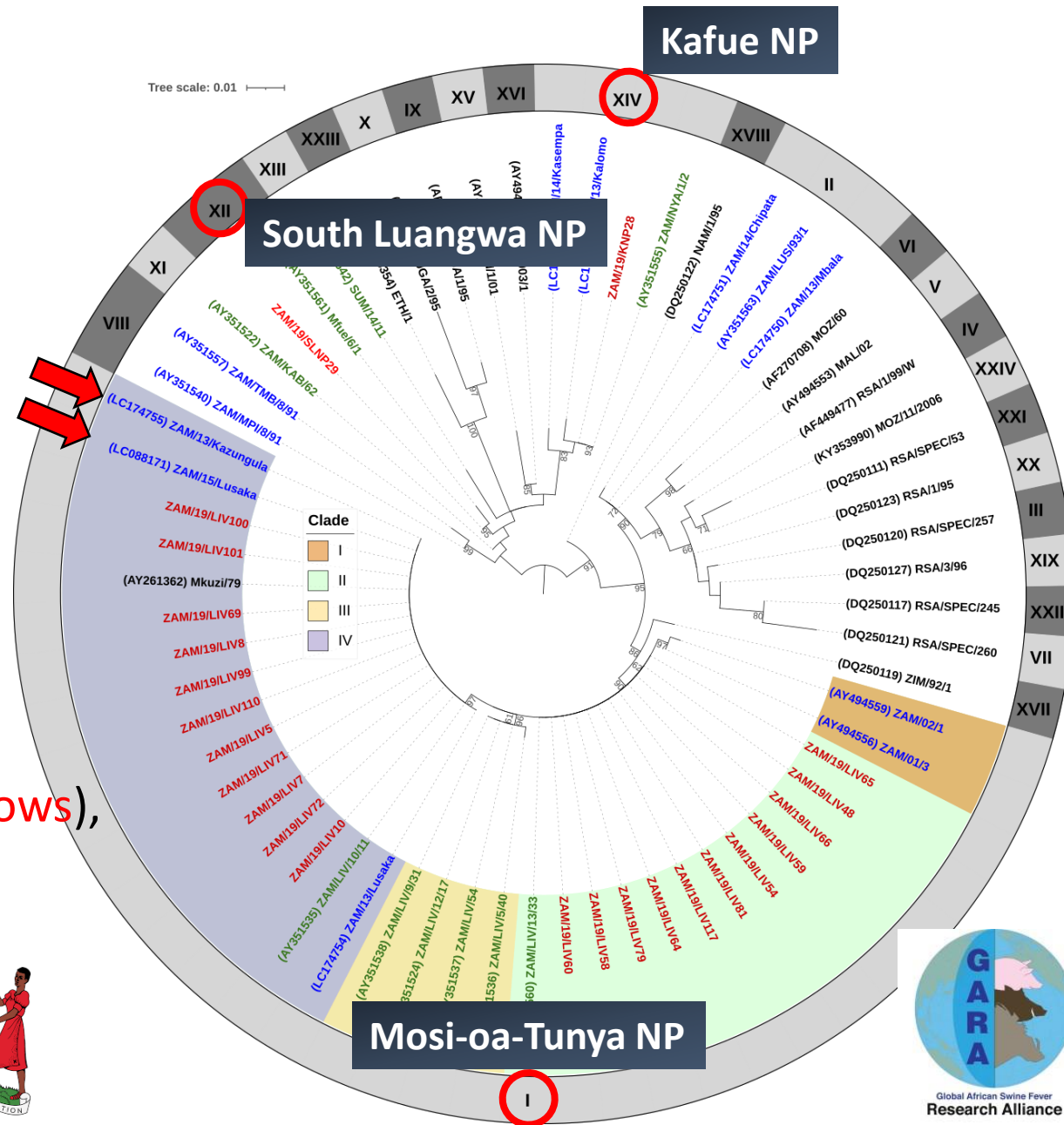
▪ **I, XII, & XIV (Red circles)**

- Genotype I: Mosi-oa-Tunya NP
- Genotype XII: South Luangwa NP
- Genotype XIV: Kafue National Park

## ○ **Zambian Genotype I viruses**

✓ Formed 4 distinct clusters, intimating high intragenotypic diversity

✓ Tick (**red text**) viruses in clade IV were 100% identical to 2013/2015 outbreak strain (**red arrows**), suggesting likely sylvatic origin







# Genetic Diversity of ASFV in Zambia – CD2v

## ASFV CD2v gene

✓ Mediates serological specificity through hemadsorption inhibition (HAI) (Malogolovkin et al., 2015: J Gen Virol)

❖ 8 serotypes have been indentified (Malogolovkin et al., 2015: J Gen Virol)

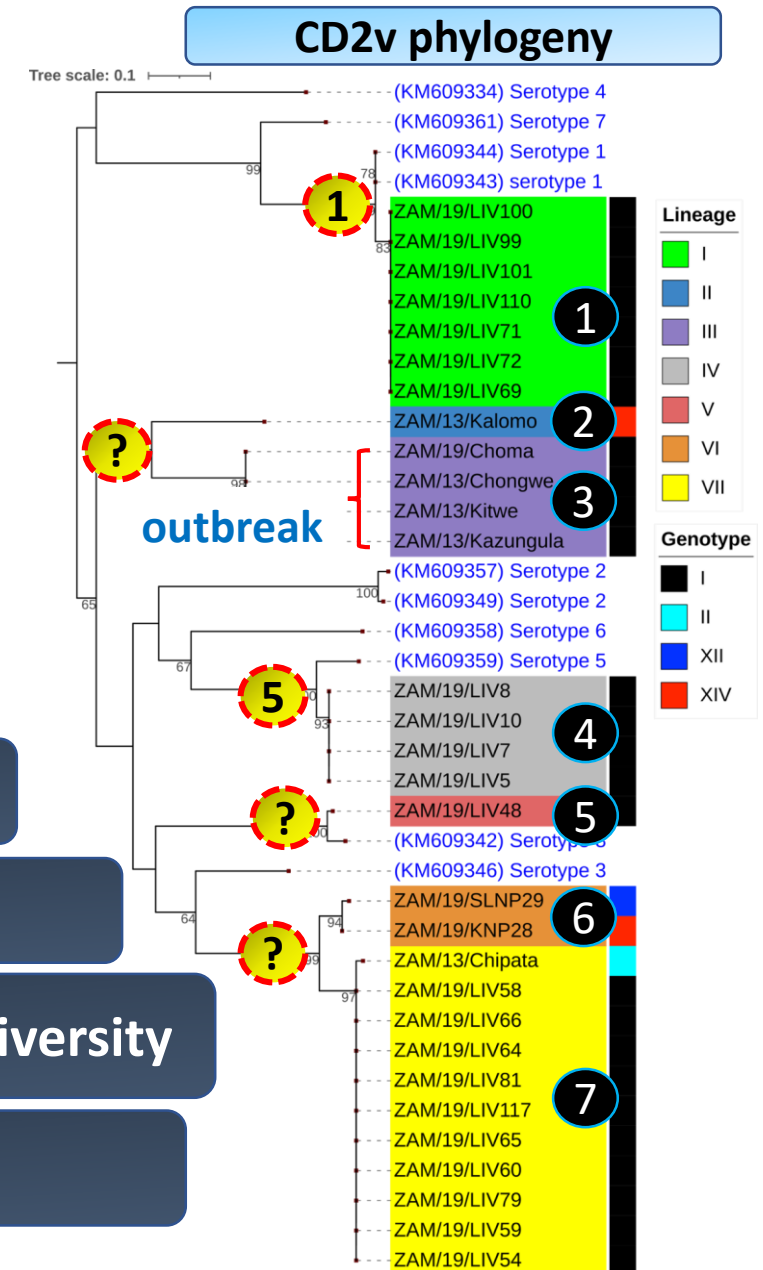
❖ HAI serotyping correlates with CD2v genotyping

✓ Some genotype I tick strains clustered with serotype 1 & 5 viruses

✓ Some viruses could not be serotyped, possibility of novel serotypes

✓ Zambian ASFVs formed 7 distinct lineages, intimating high genetic diversity

✓ Genetic diversity was more evident in genotype I strains



# Genetic Diversity of Genotype I Viruses - CVR

Strain	Genotype I CVR Variant	CVR Sequence	No. of Repeats	Host	Year of collection/Isolation
ZAM/VIC T90/1 (AM259414)	1	BNAAFNBTDBNAFF	14	Tick	1983
LIV9/31 (MF322724)	2	BNAAFNBTAFF	11	Tick	1983
ZAM/19/LIV48	3	BNAAFNBTDBNAAFNBTDBNAA	27	Tick	2019
		AAAAF			
LIV10/11 (MF322720)	4	BNAAAAAF	8	Tick	1983
ZAM/19/LIV8	4	BNAAAAAF	8	Tick	2019
ZAM/19/LIV10	4	BNAAAAAF	8	Tick	2019
ZAM/19/LIV5	4	BNAAAAAF	8	Tick	2019
ZAM/19/LIV7	4	BNAAAAAF	8	Tick	2019
ZAM/19/LIV69	5	BNAAF	5	Tick	2019
ZAM/19/LIV71	5	BNAAF	5	Tick	2019
ZAM/19/LIV72	5	BNAAF	5	Tick	2019
ZAM/19/LIV100	5	BNAAF	5	Tick	2019
ZAM/19/LIV101	5	BNAAF	5	Tick	2019
ZAM/19/LIV99	5	BNAAF	5	Tick	2019
ZAM/19/LIV110	5	BNAAF	5	Tick	2019
ZAM/13/Lusaka (LC174770)	6	BNAF	4	Pig	2013
ZAM/13/Kazungula (LC174771)	6	BNAF	4	Pig	2013
ZAM/13/Lusaka (LC174772)	6	BNAF	4	Pig	2013
ZAM/13/Choma (LC174773)	6	BNAF	4	Pig	2013
ZAM/13/Solwezi (LC174774)	6	BNAF	4	Pig	2013
ZAM/13/Kitwe (LC174775)	6	BNAF	4	Pig	2013
ZAM/15/Lusaka (LC174776)	6	BNAF	4	Pig	2013
ZAM/2002/2 (MF322710)	7	BNAFNBTDNBAG	12	Pig	2002
ZAM/1/2002/2 (MF322711)	7	BNAFNBTDNBAG	12	Pig	2002
ZAM/6/2001 (MF322713)	7	BNAFNBTDNBAG	12	Pig	2001
ZAM/4/2001 (MF322714)	7	BNAFNBTDNBAG	12	Pig	2001
ZAM/2/2001 (MF322715)	7	BNAFNBTDNBAG	12	Pig	2001
ZAM/2/2001 (MF322716)	7	BNAFNBTDNBAG	12	Pig	2001
LIV13/33 (MF359237)	8	BNADBNAFTBTDBNAF	15	Tick	1983
ZAM/19/LIV59	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV64	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV54	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV58	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV65	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV79	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV81	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV66	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV/117	8	BNADBNAFTBTDBNAF	15	Tick	2019
ZAM/19/LIV60	8	BNADBNAFTBTDBNAF	15	Tick	2019

Sylvatic origin but distinct from tick viruses

Slow/Rapid virus evolution?



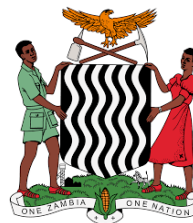
# Summary & Conclusion

## ○ This study....

- ✓ Confirmed ASFV infection in soft ticks at the wildlife-livestock interface area
- ✓ Co-circulation of multiple ASFV serotypes in sylvatic hosts and domestic pigs in Zambia
  - This finding has implications for future vaccine considerations
- ✓ High genetic diversity among ASFVs in Zambia
- ✓ High intragenotypic diversity among genotype I ASFVs in Zambia

## ○ However

- ✓ Drivers of genetic diversification among ASFVs in Zambia remain unclarified
  - Sylvatic hosts
  - Domestic pigs



# Acknowledgements

