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REGULAR ARTICLES

Ancestry and genome-wide association study of domestic pigs that survive African swine fever in Uganda

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Abstract
African swine fever (ASF) is endemic to Uganda and causes annual outbreaks. Some pigs survive these outbreaks and remain asymptomatic but are African swine fever virus (ASFV) positive. The potential heritability and genetic disparities in disease susceptibility among Ugandan pigs are not fully understood. In a 12-year study, whole blood and tissue samples were collected from 212 pigs across 19 districts in Uganda. Polymerase chain reaction (PCR) assays were used to determine ASFV infection status and genotyping was completed using a commercial porcine array. The point prevalence of ASF was calculated for each district, and breed composition origins were quantified for the sampled pigs by implementing established ancestry analyses. Genome-wide associated studies (GWAS) were conducted using all available domestic swine samples (full study population; $n=206$) as well as a reduced dataset (farm-level study population; $n=129$). This study revealed a greater number of ASFV-positive pigs in border districts than in non-border districts, a high level of admixture among domestic pigs sampled from Ugandan smallholder farms, and 48 loci that were associated with ASFV infection status. The discovery of 48 significant SNPs and 28 putative candidate genes may imply the possibility of heritability for resistance to ASFV. However, additional investigations in ASFV-endemic regions are required to fully elucidate the heritability of ASFV susceptibility among surviving pigs in Uganda.

Keywords African swine fever · GWAS · Heritability · Resistance · Susceptibility · SNPs

Introduction

African swine fever (ASF) was first discovered in *Sus scrofa domesticus* in Kenya approximately 100 years ago (Montgomery 1921). African wild swine, warthogs (*Phacochoerus africanus*) and bushpigs (*Potamochoerus porcus*), are immune to the pathogenic effects of African swine fever virus (ASFV) and present no clinical signs of disease (Anderson et al. 1998; Oura et al. 1998; Thomson et al. 1980). ASFV is highly pathogenic in European wild boar (*Sus scrofa scrofa*; Pikalo et al. 2019) and has been known to kill up to 100% of infected *S. domesticus* (Arias and Sanchez-Vizcaino 2002; Costard et al. 2013), with mortality rates varying with the presence of viral strains in circulation. In one study in Estonia, European wild boar were inoculated with ASFV (Nurmoja et al. 2017), and one survived, suggesting some resistance to ASFV in *S. scrofa*. The virulence of ASFV also seems to vary among pig breeds and endemicity of the disease in an area.

Some *Sus domesticus* breeds, especially African domestic pigs from ASFV endemic areas, have ASFV detected in their blood (based on polymerase chain reaction [PCR] assays) but are asymptomatic for the disease (Haresnape et al. 1987; Penrith et al. 2004; Uttenthal et al. 2013; Okoth et al. 2013; Muhangi et al. 2015; Thomas et al. 2016; Mujibi et al. 2018; Patrick et al. 2020). However, this trait may not be heritable. Penrith et al. (2004) reported nearly 100% mortality when the offspring of swine that had previously demonstrated resistance to ASF were challenged with

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ASFV. Some studies have argued that less virulent strains of ASF have emerged in endemic areas due to large deletions of viral genetic material encoding genes involved in modulating host immune responses (Netherton et al. 2019). A 2020 outbreak in Nigeria revealed a deletion of 6,535 base pairs between nucleotide locations 11,760 and 18,295 in the genome sequence of an ASFV of southeastern African origin and a reverse complement duplication at the 3′ end (Ambagala et al. 2023). Although the mortality rate in the Nigerian 2020 outbreak was high, reports show that mortality rates tend to decrease in ASF-endemic areas, while subclinical or chronic ASFV infections have become more frequent (Thomson 1985). However, the mechanisms responsible for the reduced viral replication and lack of disease manifestations in African *Sus scrofa* and some indigenous *S. domesticus* after ASFV infection are largely unknown. Recent studies have reported that ASFV resistance in such pigs could be due to the innate immune system of these hosts (Netherton et al. 2019; Penrith et al. 2021) and that the immune system may control viral replication, resulting in reduced systemic infection and pathogenesis. It may also be that resistance to ASFV involves a balance between virus and host factors, which have evolved over long-term infection of these hosts (Netherton et al. 2019).

In Uganda, ASFV is both endemic and enzootic, threatening the livelihood of smallholder pig producers (Muhangi et al. 2015). A 2013 study reported a high ASFV seroprevalence (53%) in apparently healthy pigs brought to a slaughterhouse in Uganda (Atuhaire et al. 2013). On the Kenya-Uganda border, ASFV DNA was detected in the tissues of two live domestic pigs (Abworo et al. 2017). Another longitudinal study in Uganda revealed three surviving pigs that were PCR positive for ASFV (Muhangi et al. 2015). Neither study found seropositive pigs, and thus, it cannot be concluded that any of the pigs had an immune response that resisted ASFV (Muhangi et al. 2015). However, it is possible that there are genomic differences in disease susceptibility among Ugandan pigs, and if this is the case, it is unknown whether such genomic differences are heritable. Ugandan pigs are thought to be a mixture of old British and modern pigs (Babigumira et al. 2021) or a mixture of Western, Far Eastern, and Indian pigs (Noce et al. 2015). However, the 'local' Ugandan pigs have not been fully characterized (Babigumira et al. 2021) and are only phenotypically described as small and black (Blench 2000). Therefore, the exact ancestry of pig breeds in Uganda is not yet fully understood. Variation in disease susceptibility among hosts is routinely investigated using genome-wide association studies (GWAS), a tool used to identify statistical associations between single nucleotide polymorphisms (SNPs) and phenotypes of interest (Visscher et al. 2017). Thus, the objectives of this study were: (1) to determine the prevalence of ASFV and the variation in the mean number of positive pigs between border and non-border districts, (2) to describe the ancestry of Ugandan domestic pigs as an underpinning of the potential for adaptation to ASFV exposure, and (3) to identify genomic regions associated with infection status among domestic pigs naturally exposed to ASFV in Uganda, using GWAS.

Materials and methods

Sample collection

Between 2010 and 2021, whole blood and tissue samples were collected from 65 smallholder pig farms, across 19 districts, in Uganda that had reported ASF outbreaks. This paper defines smallholder farmers as those with less than 2 acres of land engaged in subsistence farming and livestock (one to ten animals) rearing using family labor. The breeds represented in this study were reflective of the diverse pig production systems in Uganda. More specifically, samples were collected from 'local' Ugandan breeds, exotic breeds (e.g., commercial breeds), and local-exotic hybrids (Blench. 2000; Muhanguzi et al. 2012; Ouma et al. 2015; Twine and Njehu 2020). In most cases, the ASF outbreak was assumed to have been reported at least one week after pigs began succumbing to disease, a timeframe in which the remaining pigs were often sold in a practice referred to as *'panic selling'* (Penrith et al. 2019). Therefore, samples were collected approximately two or more weeks after the ASF outbreak began. Whole blood (from the jugular vein) in EDTA-coated vacutainer tubes or tissue (spleen and liver) in cryovials containing RNA later was collected from both asymptomatic and symptomatic pigs depending on the availability of sample type. In the field, all the samples were kept on ice packs and subsequently transferred to -20°C and −80°C freezers in the Molecular Biology Laboratory located in the Department of Environmental Management, College of Agriculture and Environmental Sciences, Makerere University, for short- and long-term storage, respectively. In total, 212 samples (whole blood, $n = 182$ and tissue, $n = 30$) were collected for the study by a project veterinarian.

DNA extraction and amplification

Total genomic DNA was extracted from both whole blood and tissue ($n = 212$). ASFV genomic DNA was extracted from 100 µl of whole blood or 10 mg of tissue, and a final eluate of 200 µl was obtained using a nucleic acid extraction kit (DNeasy Blood & Tissue Kit; QIAGEN, Germany) following the manufacturer's procedures. DNA capture was

confirmed by electrophoresis through a 2% agarose gel and visualization under ultraviolet light.

ASFV detection

Three polymerase chain reaction (PCR) assays were performed to detect the presence of ASFV in the samples: real-time ($n = 161$ samples), conventional ($n = 32$ samples), and blood ($n = 19$ samples) assays. Three different test kits were used depending on the availability of a test kit at the time of the outbreak, and the samples were made available.

Real-time polymerase chain reaction (RT–PCR)

A commercially available RTPCR kit (Tetracore Inc., Rockville, MD, USA) and Smart Cycler DX 3.0 (Cepheid, Sunnyvale, CA, USA) were used for the detection of ASFV DNA in the blood and tissue samples following the manufacturer's protocol ($n = 161$). Positive and negative controls were supplied with the RT–PCR kit.

Conventional PCR

The presence of ASFV DNA in the samples ($n = 32$) was tested via conventional PCR. The ASFV segments were amplified using the primer pair PPA1-5'-AGTTATGGGAA ACCCGACCC-3' and PPA2-5'-CCCTGAATCGGAGCAT CCT-3', which amplified a 278 bp region corresponding to the central portion of the p72 gene. PCR amplification was performed in a 25 µl reaction volume: 2 µl of the template DNA was added to 23 µl of a commercially available One *Taq* Quick-Load 2X master mix with standard buffer (New England Bio Labs Inc., UK). The cycling conditions were as follows: initial denaturation at 95 °C for 10 min for 1 cycle, denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, a final extension at 72 °C for 7 min and a hold at 4 °C. The denaturation, annealing and extension steps were repeated for 35 cycles. The PCR results were confirmed to be positive or negative by running the samples on a 2% agarose gel with a positive control sample and a marker (DNA ladder). In-house controls (positive and negative) were used. The positive control was previously purified from positive cases with sequencing data available to confirm the presence of the ASFV strain.

Blood direct

A Blood Direct PCR Master Mix Kit (Bio & SELL, Germany) was used to detect the presence of ASFV in 19 blood samples following the manufacturer's instructions. Positive and negative controls were used, and the positive control was previously purified from positive cases with sequencing data available to confirm the ASFV strain.

Statistical analysis of ASFV detection

The point prevalence of ASF was computed for each district using STATA (version 18.0; Stata Corp LLC., College Station, TX, USA, Stata Corp 2023). Point prevalence was defined as the proportion of a population of pigs in a district that had ASFV at a specific time point. The variation in the mean number of ASFV positive samples between border and non-border districts was also computed using STATA (version 18.0; Stata Corp LLC., College Station, TX, USA). Boarder districts were those located: (a) at the national border of Uganda (Arua, Busia, Isingiro, Rakai and Tororo); (b) close to the national parks (Gulu, Nwoya and Oyam); (c) with high pig density (Masaka and Mukono); and (d) hosting the national pig abattoir (capital city, Kampala). These districts received pigs (ASFV – and +) from all over the country due to trade between the capital city and other districts (Okwasimiire et al. 2023).

Genotyping

Domestic swine ($n = 212$) were genotyped using the Affymetrix Axiom Porcine Genotyping Array (658,692 single nucleotide polymorphisms [SNPs]; Thermo Fisher Scientific [Waltham, MA]; Groenen 2015) and best practices for genotype designation as defined by the manufacturer (i.e., SNP-specific priors). High-density SNPs were filtered based on the best and recommended probesets identified in the output file "recommended.ps" from the R package SNPolisher (Thermo Fisher Scientific [Waltham, MA]), 552,335 loci were retained.

Data curation

The 552,335 SNPs were mapped to the Sscrofa11.1 reference genome assembly (Warr et al. 2020) with unmapped and non-autosomal loci pruned from the dataset (53,207 loci removed). Sample and genotype quality control filters were applied using SNP & Variation Suite (SVS; version 8.9.0; Golden Helix, Bozeman, Montana, USA), which retained locus call rates > 90%, individual genotypes with > 90% call rates, and loci with minor allele frequencies (MAF) > 0.05%. No samples or SNPs were discarded due to a call rate < 0.90; however, 32,987 SNPs with an MAF < 0.05 were removed. Linkage disequilibrium pruning was performed using the composite haplotype method (window size 50, window increment 5, r^2 threshold 0.2). Following linkage disequilibrium pruning, 10,297 SNPs were retained for subsequent analyses. The R package

GENESIS ('pcair' and 'pcrelate' functions; Gogarten et al. 2019) was used to estimate degrees of relatedness (r) and evaluate duplicate samples. Both samples were discarded from the dataset when duplicated pairs ($r > 0.95$) had discordant metadata; however, one sample was retained when concordant metadata were observed. Four pairs of duplicated samples were identified (two pairs with concordant metadata and two pairs with discordant metadata), and six samples were discarded ($n = 206$).

Ancestry analysis

Ancestry was quantified for nonduplicated domestic swine genotypes ($n = 206$) using methods closely based on those detailed in Smyser et al. (2020). Briefly, ADMIXTURE (version 1.3.0; Alexander et al. 2009) was ran in a supervised framework using a *Sus scrofa* reference set comprised of 105 domestic breeds, 23 native wild boar populations, and four sister taxa. These methods deviated modestly from Smyser et al. (2020) in that the genotypes reported in this study were produced with an Affymetrix array as opposed to Illumina bead array chemistry. Accordingly, we first aligned the Affymetrix high-density genotypes to those produced with the Illumina arrays referenced by Smyser et al. (2020) with a custom R script. We then mapped loci shared across SNP genotyping platforms to the Sscrofa11.1 reference genome assembly (Warr et al. 2020). The alignment of loci across arrays provided us with 22,008 SNPs for ancestry analysis as opposed to the 29,375 loci presented in Smyser et al. (2020).

Study populations

Full study population

The full study population consisted of the 206 nonduplicated domestic swine, sampled from 65 smallholder farms across 19 Ugandan districts between 2010 and 2021. Of the 206 swine, 119 were ASFV positive (cases), and 87 were ASFV negative (controls). Samples and genotypes were quality controlled using the same thresholds described for duplicate sample removal. Briefly, only mapped autosomal SNPs from the best and recommended probesets were retained and standard quality control filters were applied using SVS (locus call rates > 90%, sample call rates > 90%, and MAF > 0.05%; version 8.9.0; Golden Helix, Bozeman, Montana, USA). Genotype quality control resulted in 466,268 SNPs for subsequent analyses. Hereafter, we use the term 'full study population' to denote the 206 domestic swine genotyped at 466,268 loci.

Farm-level study population

To ensure that the controls were selected from the same population where the cases were identified, and to minimize spurious findings due to selection biases and confounding (Zondervan and Cardon 2007; Sahana et al. 2023), smallholder farms that lacked variation in case/control status (i.e., smallholder farms in which all samples collected were ASFV positive or ASFV negative) were removed from the data. The remaining samples and genotypes were quality controlled using the same thresholds described for duplicate sample removal (locus call rates > 90%, sample call rates > 90%, and MAF > 0.05%). The resulting data consisted of 129 domestic swine sampled between 2010 and 2019 from 33 different smallholder farms across 15 districts. Approximately half of the samples were cases ($n = 65$), and half were controls ($n = 64$). Following quality control, 466,254 SNPs were retained for subsequent analyses. Hereafter, we use the term 'farm-level study population' to denote the 129 domestic swine genotyped at 466,254 loci.

Characterization of population structure

Linkage disequilibrium pruning (window size 50, window increment 5, r^2 threshold 0.2) was conducted on the full study population, using SVS (version 8.9.0; Golden Helix, Bozeman, Montana, USA), resulting in 206 samples and 10,268 loci to characterize population structure. First, we used SVS (version 8.9.0; Golden Helix, Bozeman, Montana, USA) to conduct principal component analysis (PCA; additive genetic model, markers normalized by the theoretical standard deviation under Hardy–Weinberg equilibrium [HWE]; composite haplotype method). Second, ADMIXTURE (version 1.3.0; Alexander et al. 2009) was used to evaluate K from 2 to 10 in an unsupervised framework. For each value of K, 10 iterations were conducted, and the mean and standard deviation of the cross-validation error were calculated.

Single-locus genome-wide association studies

Model selection

For both the full study population and the farm-level study population, the probability of ASFV infection status was evaluated as a function of district, ancestry composition (including Chester White, Yorkshire/Large White, and Western Heritage, as these 3 breeds represented the bulk of the ancestry associations among sampled animals), and PCR assay (RT, conventional, and blood). These evaluations were conducted using fixed effects logistic regression models (stats package [R Core Team 2021]) implemented

in the R program (version 4.1.1; R Core Team 2021). To avoid collinearity in the statistical model, pairwise correlation coefficients were calculated for the three breeds using the R package stats (R Core Team 2021). All possible combinations of fixed effects were examined using Akaike information criterion (AIC) in the R package MuMIn (Barton 2020), in which the most informative model was identified based on the lowest AIC value. Cumulative covariate weights were used to assess individual fixed effects when model uncertainty (multiple models within 2 Δ AIC of the top model) existed; weights greater than 0.5 were considered supported (Doherty et al. 2012).

EMMAX algorithm

Genome-wide association studies were performed for both study populations using the efficient mixed-model association eXpedited (EMMAX) algorithm (Kang et al. 2010) in SVS (version 8.9.0; Golden Helix, Bozeman, Montana). Identity-by-state (IBS) matrices were computed and fit as random effects in the models to account for sample stratification (underlying population structure and relatedness). Additive, dominant, and recessive models were evaluated due to uncertainty in the mode of inheritance for ASFV infection status. The pseudo-heritability (*ph*) of ASFV infection status was estimated for each model. Genomic inflation factors (λ) were calculated to evaluate how well each model accounted for the underlying population structure. Low, moderate, and high-test statistic inflation were characterized by $\lambda < 1.01$, $1.01 < \lambda < 1.05$, and $\lambda > 1.1$, respectively (Aulchenko 2015). A moderate association between ASFV infection status and a given SNP was characterized by $5 \times 10^{-7} <$ unadjusted P value $< 1 \times 10^{-5}$, and a strong association was characterized by an unadjusted P value $< 5 \times 10^{-7}$ (Consortium 2007).

Pseudo-heritability evaluation

To evaluate the probability of obtaining a pseudo-heritability estimate as or more extreme than the observed pseudo-heritability estimate, we quantified the concordance between a phenotype-based permutation distribution and a bootstrapped distribution. To generate a phenotype-based permutation distribution, the phenotypes (ASFV positive or ASFV negative) associated with individual genotypes were permuted 1,000 times, and pseudo-heritability was estimated using the EMMAX algorithm with an IBS matrix and additive model (Kang et al. 2010; http://genetics.cs.ucla.edu/emmax/index.html). The bootstrapped distribution was generated by resampling 90% of the observed data without replacement 1,000 times (sampler package in R [Baldassaro 2019]). The sample was stratified by district to ensure balanced sampling. Pseudo-heritability was estimated using the EMMAX algorithm with an IBS matrix and additive model (Kang et al. 2010; http://genetics.cs.ucla.edu/emmax/index.html) for each of the 1,000 sampled datasets. Finally, the R package bayestestR (Makowski et al. 2019) was used to calculate the overlap coefficient between the two empirical distributions.

To assess whether a given district had a significant contribution to the pseudo-heritability estimate, we used an iterative process in which individual districts were withheld and the methods described above were applied to each truncated dataset. Overlap coefficients generated from the truncated datasets were then individually compared to the overlap coefficient for the global dataset, which contained all districts. To quantify the relative contribution of each district to the pseudo-heritability estimate, we compared the bootstrapped distributions for the truncated datasets to the bootstrapped distribution for the global dataset and then subtracted the resulting overlap coefficients from one. To determine the sign of the relative contributions, the median pseudo-heritability estimate from the bootstrapped distribution for the truncated dataset was compared to the median pseudo-heritability estimate from the bootstrapped distribution for the global dataset.

Results

ASFV detection

In total, 57.8% of the samples were positive for ASFV, of which Gulu and Masaka had the highest proportions of positive samples (36.6% and 12.2%, respectively; Table 1). The mean number of positive ASFV samples in border districts was significantly greater than that in non-border districts (Student's t test: $t = 1.7854$, $p = 0.046$; Table 2).

Ancestry

At the individual level, domestic swine were highly admixed and strongly associated with reference samples from the following breeds: Chester White, Western Heritage, Yorkshire/Large White, and Landrace (Fig. 1). Across districts, associations with individual breeds varied greatly. Swine sampled in Jinja, Kampala, Lira, Lwengo, Masaka, Mpigi, and Mukono had moderate associations with Landrace, an ancestry source that was absent in Aura and Nebbi. Similarly, Western Heritage breeds represented a large contributor to domestic swine in Arua, Busia, Gulu, Isingiro, Nebbi, Oyam, Rakai, and Tororo but had minimal associations with pigs in Jinja, Luwero, Lwengo, and Mukono.

Table 1 Point prevalence of African swine fever (ASF) for 19 districts in Uganda

District	Number of positive samples	Number of negative samples	Total samples tested	Proportion of positive samples	Point prevalence (%)
Arua	3	–	3	2.4	100.0
Busia	7	–	7	5.7	100.0
Gulu	15	7	22	12.2	68.2
Isingiro	2	1	3	1.6	66.7
Jinja	2	2	4	1.6	50.0
Kalungu	1	–	1	0.8	100.0
Kampala	6	4	10	4.9	60.0
Lira	3	5	8	2.4	37.5
Luwero	3	4	7	2.4	42.9
Lwengo	–	4	4	n/a	n/a
Masaka	45	22	67	36.6	67.2
Mpigi	3	3	6	2.4	50.0
Mukono	6	3	9	4.9	66.7
Nebbi	2	3	5	1.6	40.0
Nwoya	10	10	20	8.1	50.0
Oyam	2	8	10	1.6	20.0
Rakai	1	2	3	0.8	33.3
Tororo	11	11	22	8.9	50.0
Wakiso	1	1	2	0.8	50.0
Total	**123**	**90**	**213**	**100**	**n/a**

Table 2 Variation in the mean number of African swine fever-positive samples between border and non-border districts in Uganda[a]

District category	Frequency of ASF	Mean	Standard Deviation	Upper Bound CI	Lower Bound CI
Border districts	11	9.818	12.448	1.455	18.181
Non border districts	8	1.875	1.126	0.934	2.816
All districts combined	19	6.474	10.140	1.586	11.361
Difference		7.943		-1.443	17.330

[a]Ho: diff = 0, Ha: diff > 0, Student's t test: (*t = 1.785*; *p = 0.046 and df = 17)*

Population structure

The principal component analysis provided no indication of population structure in the full study population (Fig. 2). An examination of the cross-validation error plot (Fig. 3) derived from the genetic structure analysis provided some support for 2 or 3 genetic clusters (*K*). However, further evaluation of the Q estimates from the $K = 2$ and $K = 3$ assessments were consistent with the characteristics of an uninformative model, as described by Pritchard et al. (2000), in that the majority of individuals had mixed associations with multiple clusters. Accordingly, we concluded that $K = 1$ with no ability to resolve discrete genetic clusters among the sampled animals.

Genome-wide association studies

Model selection for both the full and farm-level study populations revealed multiple competing models ($\Delta AIC < 2$); therefore, we examined the support for each fixed effect using cumulative covariate weights (Doherty et al. 2012). District, ancestry composition (Chester White, Yorkshire/Large White, and Western Heritage), and PCR assay were not informative for predicting ASFV infection status (weight < 0.50) and, therefore, were not included as fixed effects in the final model. The genomic inflation factors overwhelmingly suggested low to moderate test statistic inflation due to population stratification (Table 3). When evaluating the quantile-quantile (QQ) plots for the full study population, the observed *P* values for the additive and dominance models deviated from the expected *P* values (Fig. 4). This deviation could be caused by underlying population structure that was not sufficiently corrected for in the model or SNPs that are under selection (Ehret. 2010; Uffelmann et al. 2021). Given that three out of four methods did not indicate the presence of population structure within the data (i.e., PCA, genetic structure, and genomic inflation factors) and the lack of early separation between the observed and expected *P* values, it is plausible that these markers are under selection. Using the full study population, the additive, dominance, and recessive models identified 35, 38, and 3 significant SNPs, respectively (Table 3; Fig. 5; Supplemental Information Table S1). Thirty-three loci were shared by the additive and dominance models; thus, 43 unique loci, located on chromosomes 1, 2, 5, 6, 8, 9, 10, 11,12, 13, 14, 15, 16, and 17, were associated with ASFV infection status. The estimated pseudo-heritability for the full study population across all three modes of inheritance was 0.71 ± 0.22 (SE; Table 3). Six SNPs on chromosomes 3, 11, and 17 were moderately associated with the ASFV infection status according to the farm-level analysis (Table 3; Fig. 6; Supplemental Information Table S2). The pseudo-heritability estimate for the farm-level study population across all three genotypic models was 0.11 ± 0.30 (SE; Table 3). One intronic variant on chromosome 17 (rs340492327), was moderately associated with ASFV infection status in both study populations. Overall, 48 unique loci were associated with ASFV infection status when considering both study populations.

Pseudo-heritability

For the full study population, the overlap coefficient between the phenotype permutation distribution and bootstrapped distribution was 0.02, suggesting a true association between the ASFV phenotypes and high-density genotypes. Based on the overlap coefficients for the truncated datasets

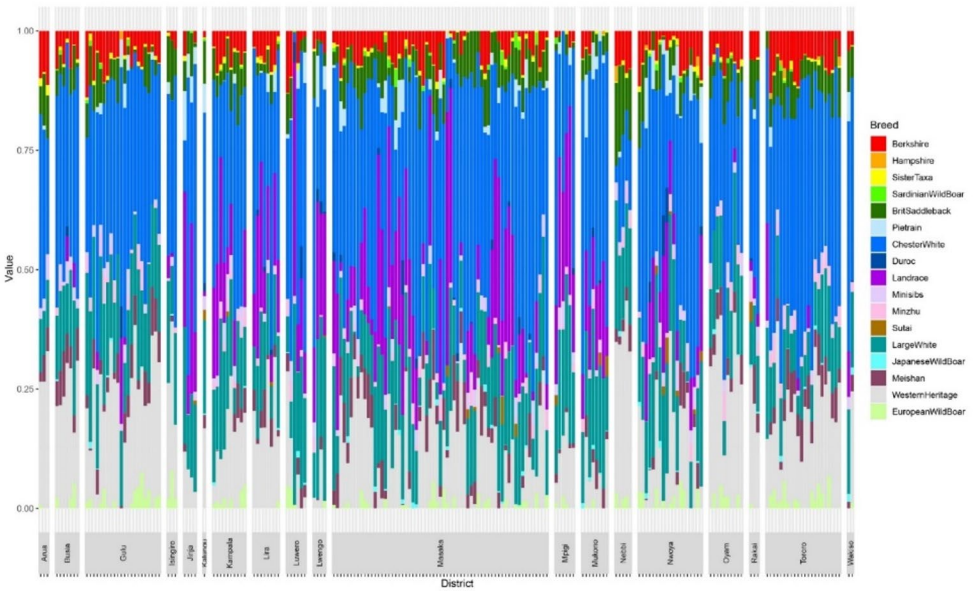


Fig. 1 Ancestry profiles for 206 domestic swine sampled across 19 districts in Uganda were generated using the methods of Smyser et al. (2020). Vertical bars represent individual domestic swine

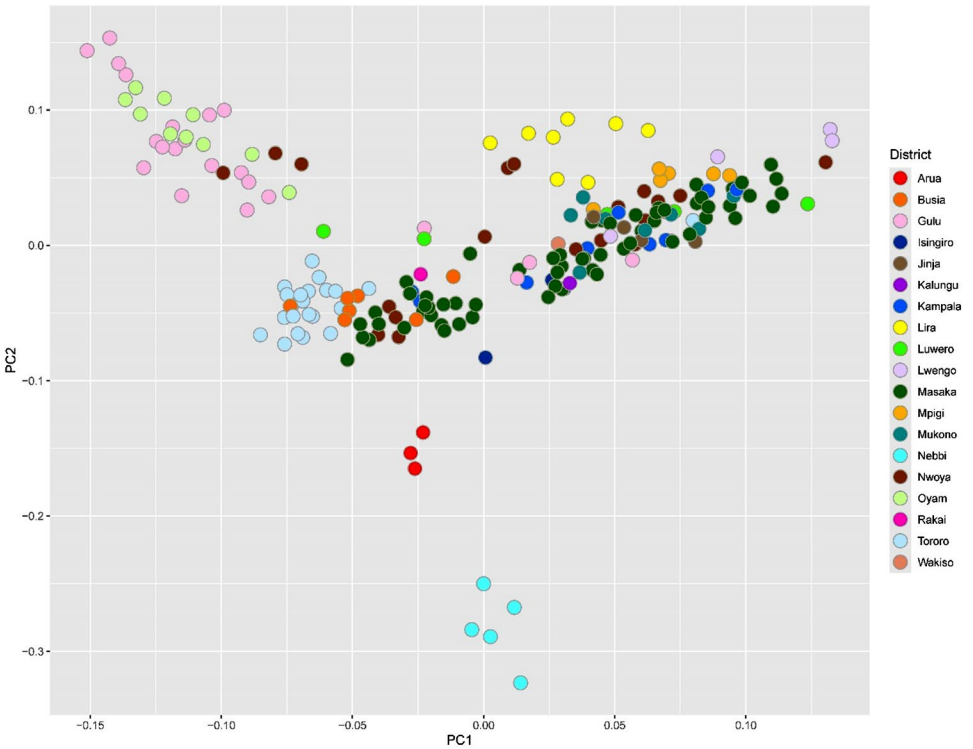


Fig. 2 Two-dimensional principal component analysis (PCA) plot for 206 domestic swine sampled across 19 districts in Uganda, color coded by district. Principal component one is represented on the x-axis and principal component two is represented on the y-axis

(Table 4), Masaka and Nwoya significantly contributed to the pseudo-heritability estimate. The relative contributions of the 19 districts in the full study population ranged from −0.80 to 0.84 (Fig. 7). The relative contribution of Masaka was 0.84, whereas that of Nwoya was 0.67. The overlap coefficient between the bootstrapped distribution and phenotype permutation distribution was 0.63 for the farm-level study population, which indicates that there is no association between genotype and ASFV infection status. Moreover, none of the districts significantly contributed to the estimated pseudo-heritability for the farm-level study population (Table 4), and the relative contribution of each district ranged from −0.35 to 0.60 (Fig. 8).

Discussion

In ASF endemic areas, such as Uganda, seemingly healthy pigs have tested positive for ASFV or ASF viral antibodies without clinical symptoms of the disease (Penrith et al.

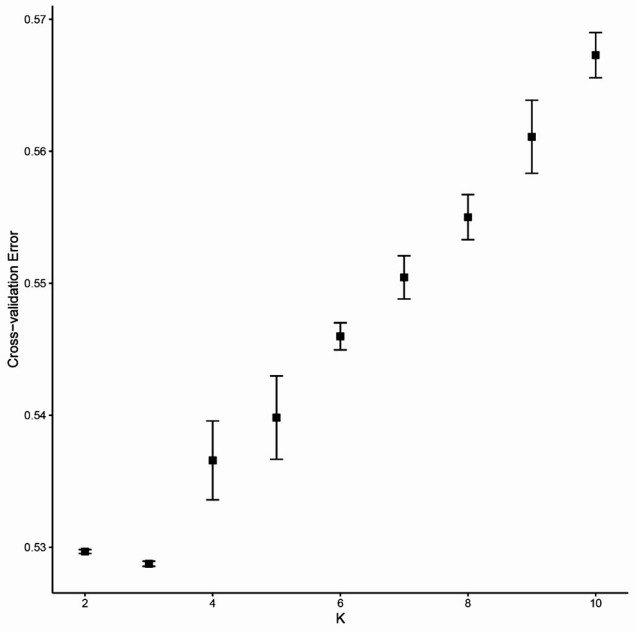


Fig. 3 Cross-validation error plot for the genetic structure analysis evaluating *K* from 2 to 10 among 206 domestic swine sampled across 19 districts in Uganda. For each value of *K*, the mean cross-validation error across 10 iterations is represented by a box with the standard deviation as a whisker

2004; Muhangi et al. 2015; Thomas et al. 2016; Patrick et al. 2020; Okwasiimire et al. 2023). As ASF continues to threaten the livelihood of swine producers within Uganda, and across the world, it is crucial to elucidate the genetic disparities among domestic swine that exhibit varied levels of susceptibility to ASFV. This study leveraged 206 domestic swine that were naturally exposed to ASFV in Uganda to compare the prevalence of ASFV between border and non-border districts, characterize the ancestry profiles of 'local' Ugandan domestic swine, and evaluate the heritability of ASFV infection status. The key findings of this study are threefold. Firstly, we found a significantly greater number of ASFV-positive pigs in border districts than in non-border districts (Student's t test: $t = 1.785$, $p = 0.046$; Table 2). Secondly, there was a high admixture of pigs among Ugandan smallholder farmers, with interdistrict variation in pig breeds. Lastly, we identified 48 loci across 15 chromosomes that were associated with ASFV infection status within naturally infected domestic swine.

African swine fever incidence

Our results revealed a significantly greater mean number of ASFV-positive pigs and a greater prevalence of ASFV in the border districts than in the non-border districts (Tables 1 and 2 respectively). The national border points, such as Arua (Uganda-Congo border), Busia and Tororo (Uganda-Kenya border), and Isingiro and Rakai (Uganda-Tanzania border), had a high prevalence of ASFV infections. Given that ASF is a transboundary disease, the high prevalence of ASF in the border districts could be explained by frequent cross-border movement associated with trade between Uganda and neighboring countries (Democratic Republic of the Congo, Kenya and Tanzania). This is further confirmed by the circulation of ASFV genotype IX in Uganda and across the border between the Democratic Republic of the Congo (Mulumba-Mfumu et al. 2019, Patrick et al. 2020), Kenya (Abworo et al. 2017) and Tanzania (Chang'a et al. 2019). The high ASFV infection rates in Masaka could be due to the high sampling frequency during this study or ecological factors arising from the high pig population density in the district. In contrast, the increase in ASFV infection in Gulu and Nwoya may be associated with their close proximity to Murchison's Falls National Park, which has a considerable population of ASFV reservoirs, such as soft ticks, bushpigs and warthogs (Kukielka et al. 2016).

Ancestry of Ugandan domestic pigs

Pigs raised on smallholder farms in Uganda were of mixed breed, with the largest ancestral contributions from Chester

Table 3 Summary of genome-wide association studies on African swine fever virus (ASFV) infection status conducted using naturally exposed Ugandan domestic swine

Study Population[a]	Mode of Inheritance	Covariates	ph[b]	SE (ph)[c]	λ[d]	Significant SNP[e]
Full	Additive	NA	0.71	0.22	1.02	35
Full	Dominance	NA	0.71	0.22	1.00	38
Full	Recessive	NA	0.71	0.22	1.00	3
Farm	Additive	NA	0.11	0.30	1.01	0
Farm	Dominance	NA	0.11	0.30	1.00	2
Farm	Recessive	NA	0.11	0.30	1.04	4

[a]Full study population (Full; $n = 206$) and farm-level study population (Farm; $n = 129$)

[b]Pseudo-heritability estimate

[c]Standard error of pseudo-heritability estimate

[d]Genomic inflation factor (λ)

[e]Number of significant SNPs identified

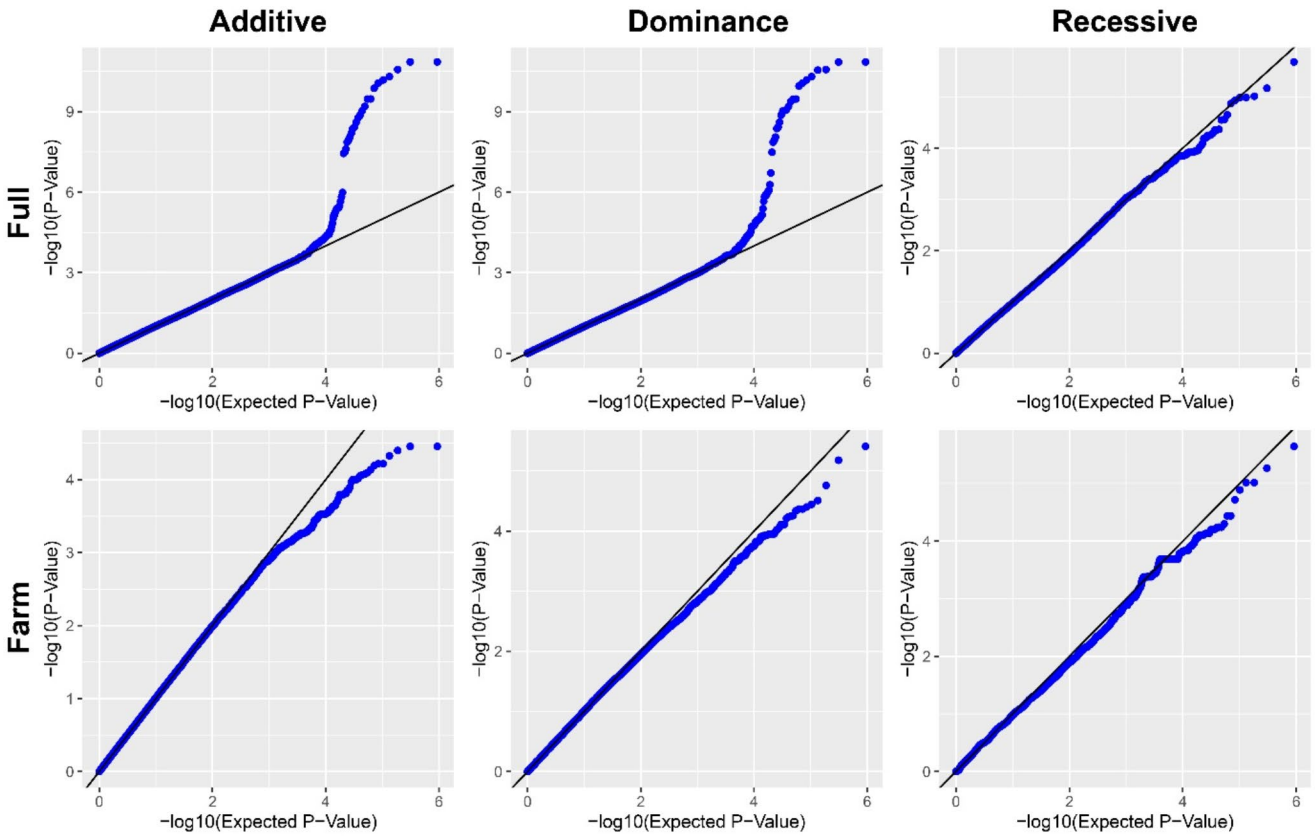


Fig. 4 Quantile–quantile (QQ) plots for African swine fever virus (ASFV) genome-wide association studies (GWAS). Two study populations (full study population [Full] and farm-level study population [Farm]) and three modes of inheritance (additive, dominant, and recessive)

White, Western Heritage, Yorkshire/Large White, and Landrace. This finding is consistent with previous studies in Uganda that reported that local smallholder pigs were a mixture of old British and modern pigs (Babigumira et al. 2021). Noce et al. (2015) reported that Ugandan pigs clustered with populations from the West (Europe/North Africa), Far East, and India. However, our study revealed additional associations to far east pigs, such as the Japanese wild boar, Sutai and Minzhu breeds of Chinese origin, and European breeds (Fig. 1). Extensive admixture among Ugandan 'local' pigs could be attributed to unregulated crossbreeding among farmers, as previously reported by Greve (2015). In Uganda, there is no government-controlled breeding program; therefore, farmers utilize crossbreeding to fulfill their goals. Some of the traits desired by Ugandan smallholder farmers include adaptive traits (disease tolerance), productive traits (fast growth rate, high birth weight and large litter size) and ease of marketing (leanness of carcass, body size and market value; Ogweng et al., unpublished). In addition, the porous borders enable traders and farmers to obtain various pig breeds from neighboring countries, further contributing to crossbreeding and the spread of transboundary livestock diseases such as ASF.

The results of this study also show interdistrict variation in breed composition origins. For example, swine sampled from the central districts of Uganda, with the exception of Lira, were moderately associated with Landrace, an association absent in Aura and Nebbi. Similarly, Western heritage breeds represented a large contributor to domestic swine in the border districts (Arua, Busia, Gulu, Isingiro, Nebbi, Oyam, Rakai, and Tororo), but had minimal associations with pigs in the districts farther from the border districts (Jinja, Luwero, Lwengo, and Mukono). The high admixture of Ugandan smallholder pigs likely occurred because of the many livestock restocking programs, by either government or nongovernmental organizations, that periodically distribute pigs of known and unknown breeds to farmers (Ampaire 2011; Amills et al. 2013; Babigumira et al., 2020; Ouma 2017; Tatwangire 2013). Additionally, the unregulated cross-border trade between Uganda and neighboring countries may have contributed to the high levels of admixture. This is supported by the variation in border district pigs from the inland districts.

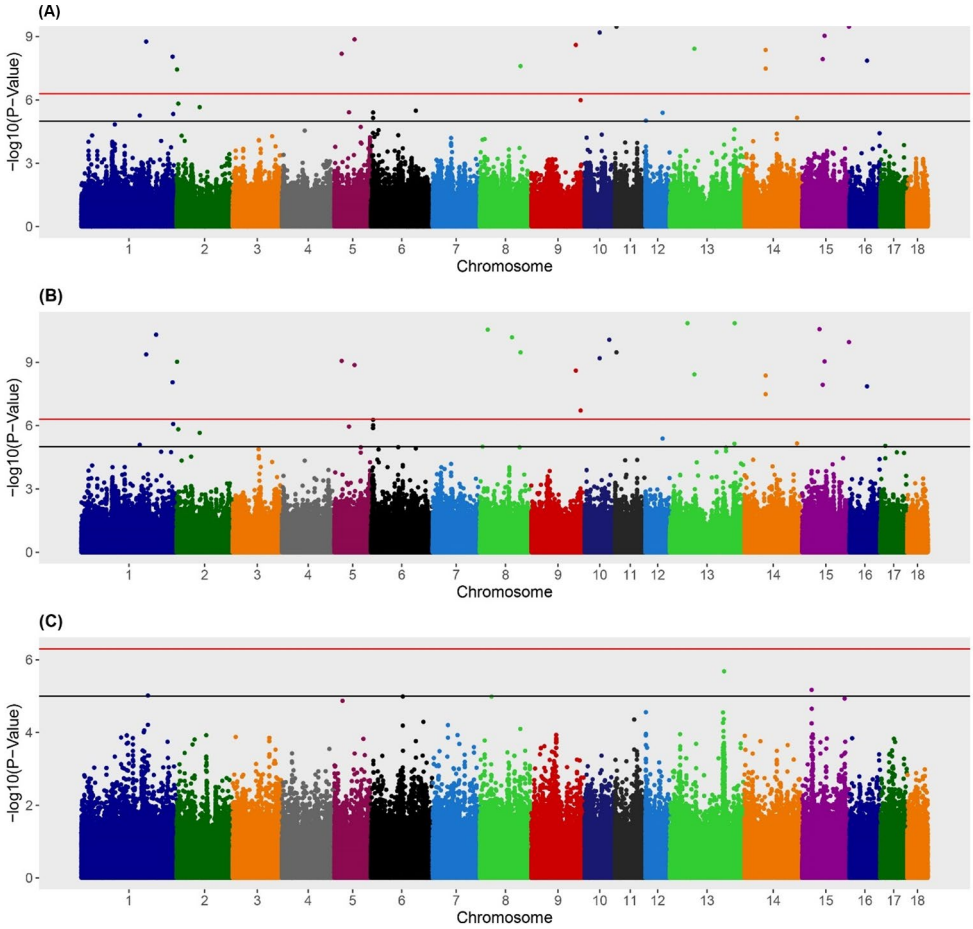


Fig. 5 Manhattan plots for African swine fever virus (ASFV) infection status in Ugandan domestic swine using the full study population ($n = 206$). (**A**) Additive model, (**B**) dominance model, (**C**) recessive model. The black line denotes a moderate association ($5 \times 10^{-7} < P$ value $< 1 \times 10^{-5}$), whereas the red line denotes a strong association (P value $< 5 \times 10^{-7}$)

Pseudo-heritability of ASFV susceptibility

The results of this study were inconclusive regarding the heritability of ASFV susceptibility. Genome-wide association studies for the full study population revealed 43 significant SNPs, whereas only 6 significant loci were identified for the farm-level study population. Moreover, the pseudo-heritability estimates for the full study population suggest that ASFV susceptibility is highly heritable ($ph = 0.71$) while the estimates associated with the farm-level study population would suggest that it is lowly heritable ($ph = 0.11$). Previous studies have reported low heritability estimates for traits that describe disease outcomes in domestic swine (Henryon et al. 2001, 2003; Bishop and Woolliams. 2010); thus, estimates from the farm-level study population better aligned with our expectations. Our inability to fully discern whether ASFV susceptibility is a heritable trait may have been influenced by several factors. For instance, we had a relatively small sample size, and we did not know exactly when the pigs were exposed to ASFV during the outbreaks or if the exposed pigs had enough time to mount an immune response and produce clinical signs before biological samples were collected. These limitations may negatively bias both SNP effect and heritability estimates (Bishop and Woolliams 2010; Bishop et al. 2012; Bishop and Woolliams 2014). Moreover, 'panic selling' of ASF survivor pigs during outbreaks, in which swine are rapidly sold for consumption, causes constant turnover of genetic resources preventing future selection of ASFV resistant animals as breeding stock (Penrith et al. 2021; Dione et al. 2014). Our findings demonstrate that the pseudo-heritability estimate was strongly affected by the inclusion of data from Nwoya and Masaka. It is plausible that the proximity of Nwoya to Murchison's Falls National Park (which is home to ASFV reservoirs such as warthogs, bushpigs, and soft ticks) and Masaka's dense pig population may explain their impact on the estimate. Targeted sampling in the districts of Nwoya and Masaka may help improve the accuracy of ASFV pseudo-heritability estimates within Ugandan domestic swine.

Disease resistance has been used in the selective breeding of livestock (Islam et al. 2020). Xu et al. (2020) reported that pigs have been genetically modified to create breeds that are immune to viral illnesses, such as porcine respiratory and reproductive syndrome virus (PRRSV), transmissible gastroenteritis virus (TGEV), and classical swine fever virus (CSFV). However, the development of ASFV-resistant

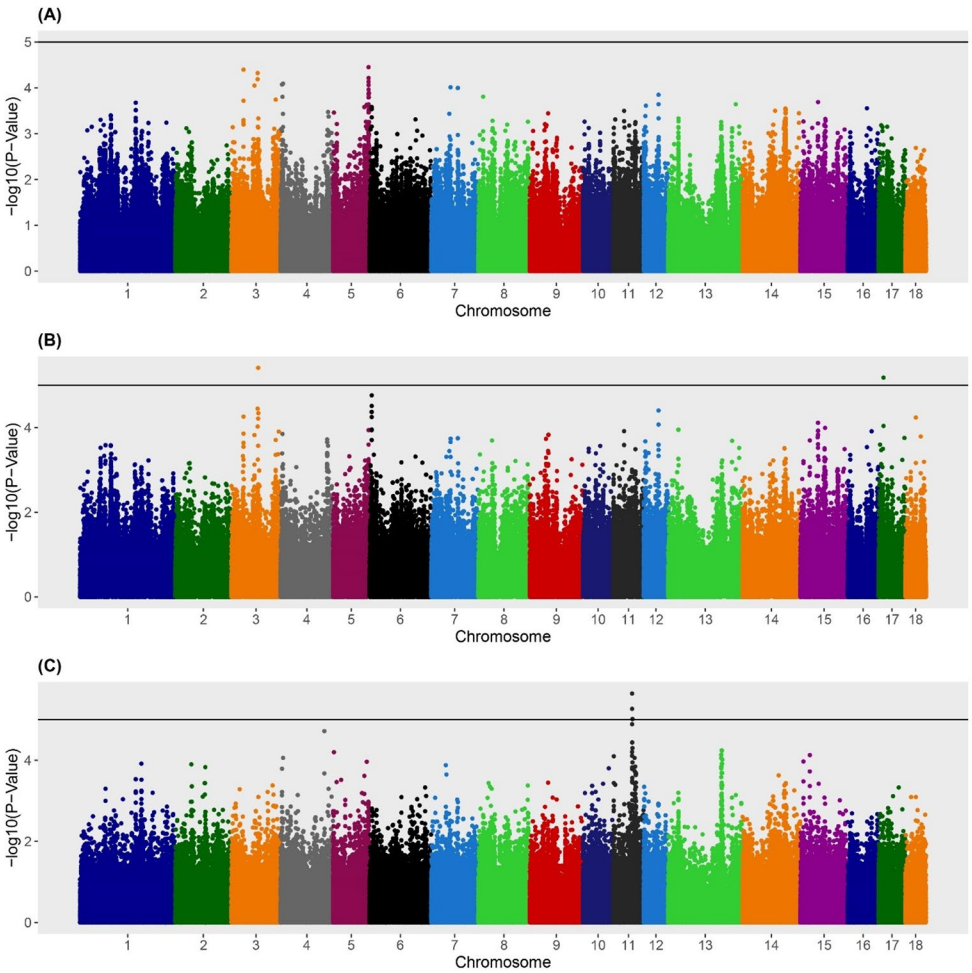


Fig. 6 Manhattan plots for African swine fever virus (ASFV) infection status in Ugandan domestic swine using the farm-level study popultion ($n = 129$). (**A**) Additive model, (**B**) dominance model, (**C**) recessive model. The black line denotes a moderate association ($5 \times 10^{-7} < P$ value $< 1 \times 10^{-5}$)

Table 4 The overlap coefficients between the phenotype permutation distributions and bootstrapped distributions were used to evaluate whether a given Ugandan district significantly contributed to the pseudo-heritability estimate for the African swine fever virus (ASFV) infection status

Dropped District	Overlap Coefficient	
	Full	Farm
Arua	0.02	-
Busia	0.02	-
Gulu	0	0.46
Isingiro	0.02	0.58
Jinja	0.01	0.47
Kalungu	0.02	-
Kampala	0.01	0.48
Lira	0.02	0.66
Luwero	0.03	0.66
Lwengo	0.03	-
Masaka	0.27	0.52
Mpigi	0.02	0.56
Mukono	0.02	0.63
Nebbi	0.02	0.45
Nwoya	0.10	0.51
Oyam	0.05	0.71
Rakai	0.01	0.69
Tororo	0.01	0.64
Wakiso	0.03	0.61

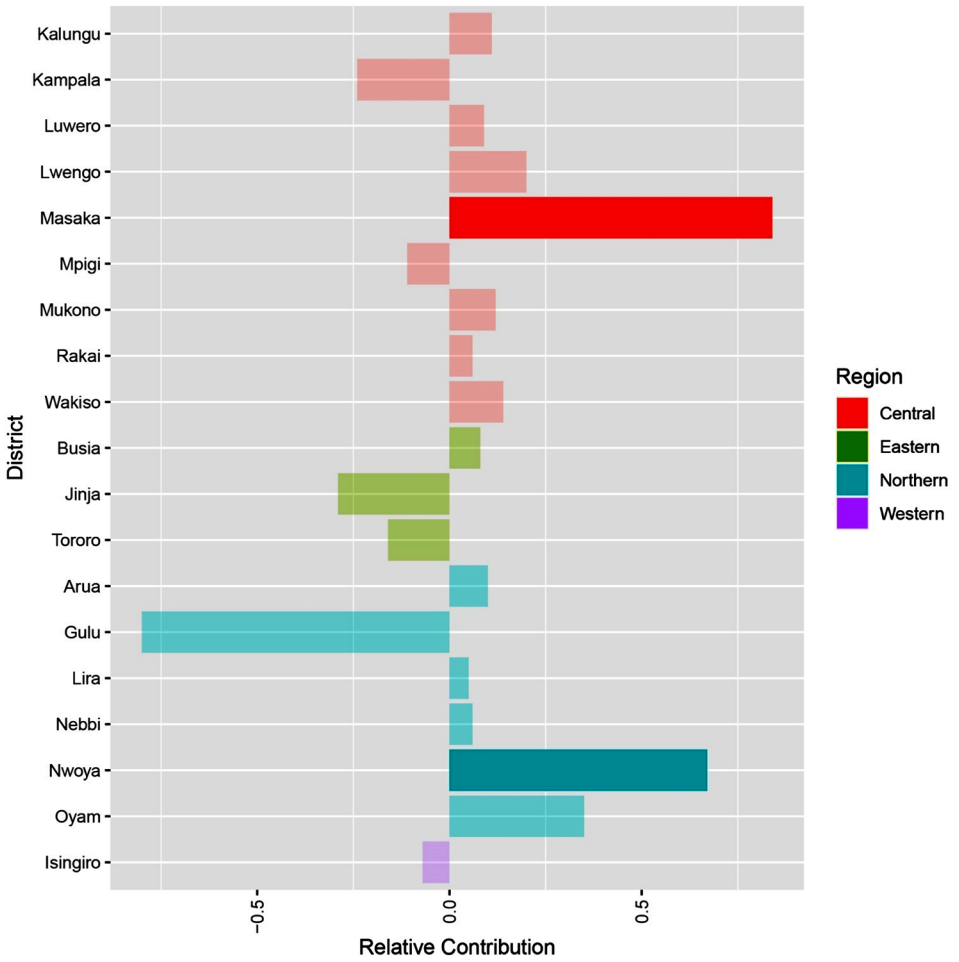


Fig. 7 Relative contribution of Ugandan districts to the pseudo-heritability estimate for African swine fever virus (ASFV) infection status generated using the full study population. Districts that are not significant contributors are represented by transparent bars

pigs through selective breeding is currently lacking. Progress has been made using warthog genomes as a model for gene editing in domestic pigs, with resistance to ASFV resulting from gene deletions, gene family contraction, and gene family expansion (Feng et al. 2023). The presence of the lactate dehydrogenase B (LDGB) gene on chromosome 5 in domestic pigs, but not on chromosome 2 in warthogs, is confirmed by genomic differences on chromosomes 2 and 5 in warthogs and domestic pigs (Feng et al. 2023). According to Feng et al. (2023), the overexpression of LDHB in warthogs may be involved in preventing ASFV proliferation and the development of viral resistance. Interestingly, the tripartite motif-containing protein family (TRIM) genes, which are reported to be contracted in warthogs (Feng et al. 2023), also seem to play vital roles in the immune response to pathogens (Reymond et al. 2001).

In experimentally ASFV-inoculated domestic pigs and warthogs, the TRIM family of genes expanded in the susceptible Duroc, but contracted in the warthog and Kenyan domestic pig breeds with reported ASFV resistance (Feng et al. 2023) and seemed to play a vital role in the immune response to pathogens (Reymond et al. 2001). According to Palgrave et al. (2011), the ReLA gene, which is likewise found on chromosome 2, is considered to contribute to the resistance of warthogs to ASFV by controlling their immune response to infections. Nevertheless, after being exposed to a moderately virulent ASFV, some domestic pigs whose ReLA gene was edited to contain all three amino acids found in the warthog RELA (Lillico et al. 2016; McCleary et al. 2020) were not resistant to ASF (Feng et al. 2023) but showed a delay in the onset of clinical signs. In-depth investigation of the functions of the LDHB, TRIM, and RelA genes in warning of ASFV resistance and alterations in these genes in domestic pigs may be essential for managing ASFV (McCleary et al. 2020). A detailed exploration of the roles of these three genes (LDHB, TRIM and RelA genes) in ASFV resistance in warthogs and the modification of these genes in domestic pigs may be vital for controlling ASFV.

Our study identified 48 SNPs that were moderately or strongly associated with ASFV infection status. The majority of the loci, including rs340492327, were intronic; however, approximately one third of the SNP were intergenic (Supplemental Information Tables S3 and S4). In addition,

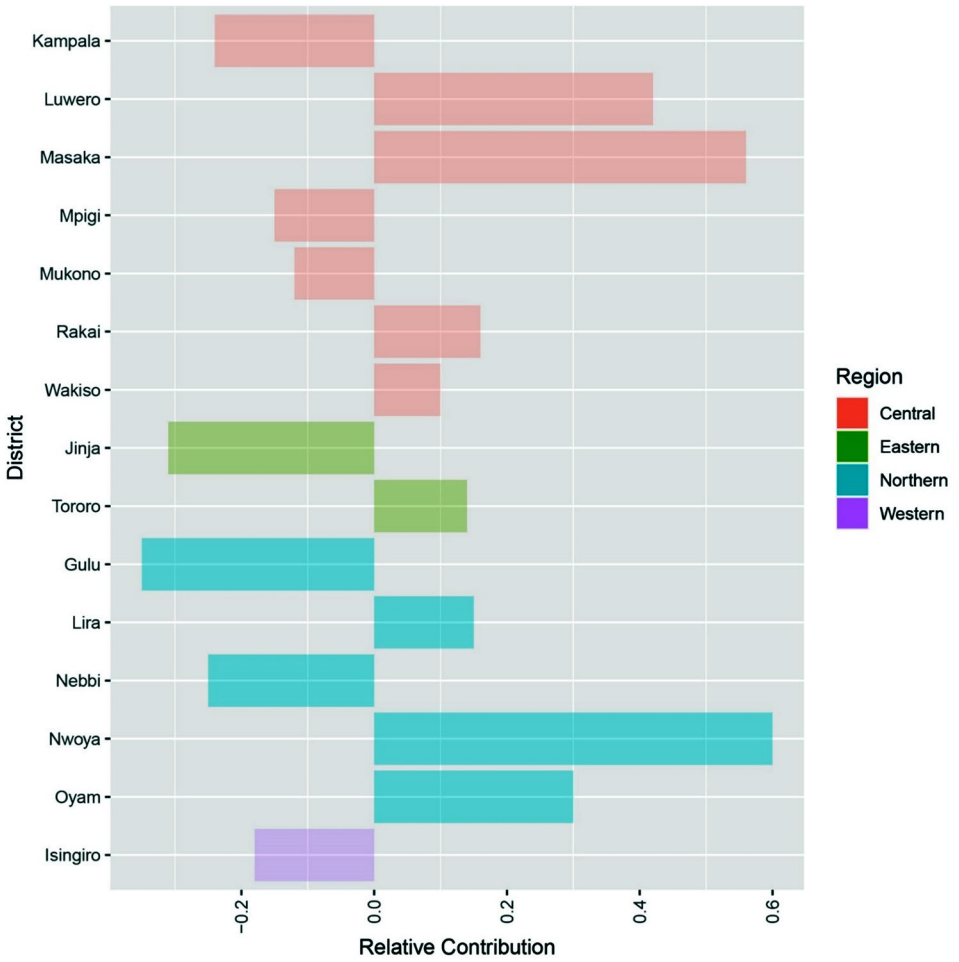


Fig. 8 Relative contribution of Ugandan districts to the pseudo-heritability estimate for African swine fever virus (ASFV) infection status generated using the farm-level study population. Districts that are not significant contributors are represented by transparent bars

several loci were classified as upstream or downstream gene variants. Twenty-eight putative candidate genes were associated with the 48 significant loci (Supplemental Information Tables S3 and S4). This finding, in addition to the pseudo-heritability estimates, suggests that ASFV susceptibility may be heritable among Ugandan pigs that survive ASF outbreaks. However, further research in ASFV endemic areas in Africa is needed to fully understand the heritability of ASFV susceptibility.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11250-024-04195-5.

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Author contributions Peter Ogweng, Vincent B. Muwanika, Antoinette J. Piaggio and Charles Masembe conceptualized and designed the study; Peter Ogweng and Charles Masembe collected the samples; Courtney F. Bowden and Timothy J. Smyser analyzed the SNP data; all the authors drafted and critically revised the manuscript; Charles Masembe and Antoinette J. Piaggio obtained the funding. All of the authors have read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate The Ugandan National Council for Science and Technology approved the study under reference number A497. We also obtained consent from district veterinary officers and informed consent from farmers before sampling pigs from their districts and farms, respectively.

Conflict of interest The authors declare no conflicts of interest.

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