ENVIRONMENTAL ASSESSMENT

In support of approval of the original application for the deletion of exon 7 of the *CD163* gene that, in the homozygous lineage of domestic pigs (*Sus scrofa)*, is intended to confer resistance to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV).

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TABLE OF CONTENTS

LIST OF ACRONYMS 3

TECHNICAL TERMS 4

1. EXECUTIVE SUMMARY 6

2. INTRODUCTION 7

- 2.1 PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS 7
  - 2.1.1 TAXONOMY AND ORIGIN 7
  - 2.1.2 TRANSMISSION 7
  - 2.1.3 CLINICAL SIGNS 9
- 2.2 PURPOSE AND NEED FOR THE PROPOSED ACTION 10
- 2.3 APPROACH TO RISK ASSESSMENT 10

3. DESCRIPTION OF PRODUCT 11

- 3.1 PRODUCT DEFINITION AND DESCRIPTION 11
- 3.2 PRODUCTION SCHEME 11
- 3.3 MOLECULAR CHARACTERIZATION 13
- 3.4 PHENOTYPIC CHARACTERIZATION 14
- 3.5 GENOTYPIC AND PHENOTYPIC DURABILITY 14
- 3.6 EFFECTIVENESS/CLAIM VALIDATION 15

4. BACKGROUND ON DOMESTIC PIG PRODUCTION AND WILD PIG POPULATIONS IN THE UNITED STATES 15

- 4.1 PIG FARMING PRACTICES IN THE UNITED STATES 15
  - 4.1.1 REPRODUCTION AND BREEDING 15
  - 4.1.2 DISTRIBUTION AND TYPES OF PIG FARMS IN THE UNITED STATES 16
  - 4.1.3 CONTAINMENT ON PIG FARMS 18
  - 4.1.4 DISPOSAL OF PIG CARCASSES AND WASTE 21
  - 4.1.5 TRANSPORTATION OF DOMESTICATED PIGS IN THE UNITED STATES 21
- 4.2 WILD PIGS IN THE UNITED STATES 21

5. AFFECTED ENVIRONMENT 25

6. RISK CHARACTERIZATION 26

- 6.1 EVALUATION OF THE FITNESS OF PIGS WITH THE IGA 26
- 6.2 LIKELIHOOD OF EXPOSURE 28
  - 6.2.1 ESCAPE 28
  - 6.2.2 SURVIVAL AND DISPERSAL 30
  - 6.2.3 REPRODUCTION AND ESTABLISHMENT 32
  - 6.2.4 CONCLUSION ON EXPOSURE 34
- 6.3 LIKELIHOOD OF HARMS RESULTING FROM EXPOSURE 34
- 6.4 SIMULATION MODELING 37
- 6.5 POTENTIAL TOXICITY DUE TO THE IGA 38
- 6.6 RISK OF HARM TO THE ENVIRONMENT 38

7. ALTERNATIVE TO THE PROPOSED ACTION 40

8. AGENCIES AND PERSONS CONSULTED 40

9. LIST OF PREPARERS 40

10. REFERENCES 41

LIST OF TABLES

TABLE 4-1. PERCENTAGE OF PIGS BY FACILITY TYPE AND PHASE FOR LARGE FARMS (⩾ 100 PIGS) (USDA, 2015). VALUES IN PARENTHESES ARE STANDARD ERRORS. 18
TABLE 4-2. PERCENTAGE OF SMALL FARMS (< 100 PIGS) CONTAINING WEANED PIGS AND SOWS/GILTS BY FACILITY TYPE AND PHASE (USDA, 2014). VALUES IN PARENTHESES ARE STANDARD ERRORS. 19
TABLE 6-1. ESTIMATED PREVALENCE OF PRRSV IN WILD PIG POPULATIONS IN THE UNITED STATES 27

LIST OF FIGURES

FIGURE 3-1. COMMERCIAL BREEDING STRATEGY FOR PRRSV-RESISTANT PIGS. 13
FIGURE 4-3. ESTIMATED PROPORTION OF ALL DOMESTICATED PIGS IN THE UNITED STATES, WITH AND WITHOUT OUTSIDE ACCESS, FOR SMALL (<100 PIGS) AND LARGE-ENTERPRISE (⩾100 PIGS) FARMS BASED ON DATA FROM THE 2017 USDA CENSUS OF AGRICULTURE AND USDA PIG OPERATION SURVEYS (USDA, 2014, 2015). EACH GRID CELL REPRESENTS 100,000 PIGS. 20
FIGURE 4-4. CHANGE IN DISTRIBUTION OF WILD PIG POPULATIONS FROM 1982-2022 (USDA, 2023). 23
FIGURE 4-5. MAP OF PREDICTED WILD PIG DENSITIES FOR 2021 24
FIGURE 6-1. PUNNETT SQUARES SHOWING THE EXPECTED MENDELIAN INHERITANCE OF IGA IN F1 AND F2 OFFSPRING THAT WOULD BE PRODUCED FROM ESCAPED IGA FEMALES 28
FIGURE 6-2. U.S. 2022 BILLION-DOLLAR WEATHER AND CLIMATE DISASTERS. REPRODUCED FROM NOAA (2023). 29
FIGURE 6-3. OVERVIEW OF THE MULTIPLE CONDITIONS REQUIRED BEFORE THE IGA CAN BEGIN TO PROVIDE A SUBSTANTIVE FITNESS ADVANTAGE TO WILD PIG POPULATIONS. 39

LIST OF ACRONYMS

AI	Artificial Insemination
DNA	Deoxyribonucleic acid
EA	Environmental Assessment
E0	First generation
F1	First generation offspring
F2	Second generation offspring
FD&C	Food Drug & Cosmetic
FDA	U.S. Food and Drug Administration
FOI	Freedom of Information
FONSI	Finding of No Significant Impact
GC	Guanine/Cytosine
GE	Genetically Engineered
GWAS	Genome-Wide Association Studies
IFN	Interferon
IGA	Intentional Genomic Alteration
LDV	Lactate Dehydrogenase-elevating Virus
MSE	Mean Squared Errors
NRC	National Research Council
NEPA	National Environmental Policy Act
PEARS	Porcine Epidemic Abortion and Respiratory Syndrome
PIC	Pig Improvement Company (parent organization is Genus plc)
PiGMaP	Pig Gene Mapping Project
PQA	Pork Quality Assurance
PRRS	Porcine Reproductive and Respiratory Syndrome
PRRSV	Porcine Reproductive and Respiratory Syndrome Virus
PRV	Pseudorabies Virus
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
sd	Standard Deviation
sgRNA	Single Guide RNA
SIRS	Swine Infertility and Respiratory Syndrome
SNP	Single Nucleotide Polymorphism
SpyCas9	Cas9 nuclease from *Streptococcus pyogenes*
SRCR5	Scavenger Receptor Cysteine-Rich domain 5
USDA	United States Department of Agriculture

TECHNICAL TERMS

Allele (NIH, n.d.)	A form of one or more alternative versions of a genetic region (e.g., gene) within a genome.
Boar (Hoar, n.d.)	Intact, sexually mature, male pigs intended for breeding purposes.
Cas9 protein-guide RNA complex (Asmamaw, 2021)	A method of introducing an alteration into the DNA. A guide RNA is synthesized to locate and bind to a target sequence in the DNA. The guide RNA is made up of bases that complement the target DNA sequence in the genome, therefore, the guide RNA should only bind to the target sequence and no other regions of the genome. Cas9 is an enzyme that follows the guide RNA to the same location in the DNA sequence and makes a cut across both strands of the DNA.
Chromosome (NIH, n.d.)	Threadlike structure composed of protein and DNA within the cells of an organism that serve to carry genomic information from cell to cell.
Exon (NIH, n.d.)	The portion of a gene that will be combined with other exons to form the final mature RNA.
Farrowing (Hoar, n.d.)	The process of giving birth to piglets.
Feral pig (VerCauteren, 2019)	Wild pig that has escaped or is descended from an escaped domestic pig.
Fitness (NRC, 2002)	A measure of an individual’s survival and reproductive success (e.g., number of offspring).
Genome (NIH, n.d.)	The entire set of genetic instructions found in a cell.
Genotype (World Library of Science, n.d.)	Genetic makeup of an individual, usually with reference to a specific combination of alleles.
Gilt (Hoar, n.d.)	Female pigs intended for slaughter or breed that have not yet farrowed a litter.
Homozygous (NIH, n.d.)	The presence of two identical alleles at a particular gene locus (e.g., AA).
Homozygous null	An animal without the IGA resulting from the mating of an animal homozygous for the IGA and a animal without the IGA or from the mating of two animals heterozygous for the IGA.
Homozygous recessive phenotype (NIH, n.d.)	Phenotype (trait) that emerges only when individuals are homozygous; heterozygous individuals do not exhibit the phenotype.

Heterozygous (NIH, n.d.)	The presence of two different alleles at a particular gene locus (e.g., Aa).
IGA/IGA genotype	Homozygous state of the IGA (individual with two copies) that confers resistance to PRRSV.
Introgression (Twyford, 2012)	The transfer of genetic information from one species to another as a result of hybridization between them and repeated backcrossing.
Locus (NIH, n.d.)	Specific physical location of a gene or other DNA sequence withing a genome.
Neutral genetic variation (NIH,2007)	Genetic variation that provides no fitness advantage or disadvantage.
Phenotype (NIH, n.d.)	The set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.
Plasticity (Sommer, 2020)	The property of organisms to produce distinct phenotypes in response to environmental variation.
Sow (Hoar, n.d.)	Female pig that has farrowed at least one litter of piglets.
Wild pig (VerCauteren, 2019)	Any free ranging suid (pig). Also referred to as a feral pig in this document.

1. EXECUTIVE SUMMARY

This document constitutes an Environmental Assessment (EA) of an intentional genomic alteration (IGA) present in domestic pigs (*Sus scrofa*) designed to confer resistance to the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV). PRRSV, the causative agent of the Porcine Reproductive and Respiratory Syndrome (PRRS) disease, is a significant etiological agent in the pig industry worldwide. The IGA is the deletion of exon 7 in the porcine gene encoding CD163, which is the primary binding target for PRRSV. The deletion is intended to confer resistance to PRRSV Type 1 and Type 2 in the homozygous animals, known as PRRSV-Resistant Pigs. This EA addresses requirements stipulated under the National Environmental Policy Act (NEPA) and in United States Food and Drug Administration (FDA) regulations (21 CFR Part 25 and 21 CFR 514.1(b)(14)) to determine whether the agency action (i.e., approval of the article, the IGA) is likely to have a significant impact on the human environment in the United States.

Risk in this assessment was evaluated as the product of the probability of exposure and the conditional probability of environmental harm assuming exposure to the IGA occurs. Given the existing adverse ecological impacts that invasive wild pigs have in North America, this evaluation primarily focused on the potential for pigs with the IGA to escape, survive and reproduce with wild pig populations, potentially resulting in establishment of wild pig populations containing the IGA. In theory, if the IGA imparts a fitness advantage to wild pigs by causing them to be resistant to PRRSV, improving survival and reproduction in wild pig populations, it could potentially result greater invasiveness of wild pigs and greater environmental harm. In contrast, if the IGA imparts little to no fitness advantage to wild pigs, then the environmental harm of pigs with the IGA would not be any different than domestic pigs without the IGA. Thus, this evaluation focused on whether pigs with the IGA pose any more environmental risk than domestic pigs without the IGA.

Based on the planned production scheme and proposed approval conditions for the product, the potentially affected environment includes the entire United States because pigs with the IGA could be reared on any farm. Only pigs that are homozygous for the IGA are resistant to PRRSV; heterozygous pigs are not resistant. However, farms could contain a mixture of genotypes at any one time, including domestic pigs without the IGA, pigs heterozygous for the IGA (not PRRSV-resistant) and pigs homozygous for the IGA (PRRSV-resistant). Thus, all potential genotypes were considered in this evaluation. For the purposes of this document, the terms “pigs with the IGA” refers to heterozygous and/or homozygous pigs, and “PRRSV-Resistant pigs” refers to pigs homozygous for the IGA. In addition, because there are no specific containment requirements proposed for the pigs with the IGA, this evaluation conservatively assumes that pigs with the IGA will enter the environment.

Other than resistance to PRRSV, there are no phenotypic differences between pigs with the IGA and domestic pigs without the IGA. Thus, pigs with the IGA are not expected to be more likely to escape or disperse compared to pigs without the IGA, but pigs with the IGA could have greater likelihood of survival and reproduction (i.e., improved fitness) in the environment when infected with PRRSV compared to domestic pigs without the IGA. However, based on the low recorded prevalence of PRRSV in wild pig populations (ranging from 0-3.4%), the IGA is expected to impart little to no fitness advantage to pigs with the IGA or wild pigs infected with PRRSV under natural conditions. Because the IGA is homozygous recessive, if it entered wild pig populations, the IGA prevalence is expected to generally decrease in frequency over time under most conditions but may persist at very low frequency for decades. In such cases that the IGA persists, the prevalence of homozygous individuals (IGA/IGA genotype) is expected to remain extremely low in most populations. Simulation modeling corroborates these conclusions and suggests that even under a broad range of conservative representative conditions (i.e., reasonable conservative and reasonable worst-case scenarios), the introgression of the IGA into wild pig populations is not expected to increase the growth of wild pig populations. Consequently, there is no indication that pigs that contain the IGA present a greater likelihood of exposure or harm to the environment than domestic pigs that do not contain the IGA. Therefore, pigs with the IGA do not pose any more risk to the environment than domestic pigs without the IGA, and no significant impacts to the human environment from approval of the IGA in pigs are expected.

2. INTRODUCTION

Domestic pigs (*S. scrofa),* commonly known as swine, hogs, or simply as pigs, are raised in the United States, and throughout the world, as an important source of meat for human and animal consumption. They are characterized by different names based on the sex and developmental stage of the animal. Boar and sire refer to uncastrated mature male pigs, whereas sow is the term used to describe females that have farrowed or given birth to piglets. Gilt is a term used to denote a mature female pig under the age of 1 year prior to her first farrowing. Domestic pigs are diploid (2n = 38) with haploid chromosome numbers (n = 19) found in the gametes. The genetic makeup of the domestic pig is organized on 18 pairs of non-sex chromosomes (autosomes) and 1 pair of sex chromosome (allosomes).

2.1 Porcine Reproductive and Respiratory Syndrome Virus

2.1.1 Taxonomy and origin

PRRSV is an enveloped, single-stranded RNA virus within the family *Arteriviridae* (Nelsen et al., 1999). The first documented cases of PRRSV were in the late 1980s in the United States and Germany, manifesting as severe reproductive and respiratory disease in pigs. PRRSV was originally referred to as swine infertility and respiratory syndrome (SIRS) and porcine epidemic abortion and respiratory syndrome (PEARS) (Leedom Larson, 2016a). The origin of PRRSV is unknown and the virus is believed to have been circulating long before it was first described (Shi et al., 2010). There are two primary strains of PRRSV. Type 1 is a European strain and Type 2 is a North American strain (Leedom Larson, 2016a).

2.1.2 Transmission

Pigs are the only known hosts for PRRSV and there is no evidence that the virus is zoonotic (i.e., spread between people and animals) (Leedom Larson, 2016a). Current understanding of PRRSV transmission in pigs is based primarily on studies of farm populations. Transmission within wild populations and between farm and wild populations remains poorly understood (R. Miller, personal communication, 2022).

PRRSV is transmitted directly via intranasal, oral, intrauterine/transplacental, and vaginal routes through contact with bodily fluids such as nasal secretions, blood, saliva, urine, semen, mammary secretions, and feces (Zimmerman et al., 2019a). The most effective route of transmission appears to result from activities that lead to breaks in the skin, such as husbandry practices (e.g., ear notching, tail docking), injection, bites, or scrapes (Leedom Larson, 2016a).

Aerosol transmission has also been documented, along with indirect transmission via exposure to fomites[1] or insect vectors such as houseflies and mosquitoes (Zimmerman et al., 2019a).

Once PRRSV is transmitted to the tonsil or upper respiratory system, it enters the cell via glycoprotein receptor sialoadhesin and the transmembrane protein CD163 found on macrophages where it replicates and spreads to lymphoid tissues (spleen, thymus, tonsil, lymph nodes), lungs, and other tissues (Zimmerman et al., 2019a). PRRSV can cross the placenta to fetuses after 72 days of gestation (Iowa State University, 2023).

PRRSV infection leads to inhibition of type I interferons (IFN) and suppresses innate immunity in infected pigs (Sun et al., 2012). Infection results in cell-mediated immune responses (e.g., production of neutralizing antibodies against the viral proteins N and GP5, and NSPs 1, 2, and 7) that peak within ~10 weeks of infection (Lunney et al., 2016). Viral shedding usually ends within 60 days of infection and is accompanied by a decrease in antibody titers over a period of 4-8 months; however, shedding can persist for a longer period in some pigs (Iowa State University, 2023). Piglets can benefit from passive immunity, but this declines soon after weaning and weaned pigs are typically much less resistant to PRRSV than adult animals (Zimmerman et al., 2019a). Herds can be simultaneously infected with more than one PRRSV strain, and on the individual level, infection with one PRRSV strain does not necessarily prevent infection from other strains (Leedom Larson, 2016a). Factors influencing transmission include the age of pigs at the time of infection (younger pigs have greater viral loads), variation in the virulence of PRRSV isolates, and immune response (Pileri & Mateu, 2016).

Within-farm transmission occurs primarily through vertical transmission via parent to susceptible offspring and comingling of infected and susceptible pigs, whereas between-farm transmission occurs primarily through exposure to infected pigs, contaminated semen, or infective aerosols (Zimmerman et al., 2019a). The introduction of infected pigs is the primary cause of herd-to-herd transmission, however contaminated semen and fomites can also play a major role in transmission (Zimmerman et al., 2019b). Risk of transmission between farms has also been shown to be positively related to proximity of infected farms (Zimmerman et al., 2019a), but does not appear to depend on farm size, and the non-animal/human means of spread in these cases is poorly understood (Reiner et al., 2009).

Based on the low prevalence of PRRSV in wild populations (Albina, 2000; Pedersen et al., 2018; Ruiz-Fons et al., 2008) and the similarity between the type of PRRSV identified in wild populations and commercial live vaccines (Pileri & Mateu, 2016), transmission is believed to occur from farm-to-wild populations rather than from wild-to-farm populations. There is no evidence that the wild population is a reservoir for PRRSV, further indicating that farm populations are acting as a source of reintroduction of the virus to wild populations (Ruiz-Fons et al., 2008). A 2018 study that investigated the presence of PRRSV antibodies in wild pig populations in the United States found a positive association between the number of small farms (but not large farms) in a state and the prevalence of PRRSV in the wild population (Pedersen et al., 2018). Within a state, every increase in 100 small farms was associated with an 11% increase in prevalence of PRRSV in wild pig populations; however, the measured prevalence never exceeded 3.4%. Studies in Europe, however, have found only weak relationships between the prevalence of PRRSV in wild populations and farm populations (Reiner et al., 2009; Rodríguez-Prieto et al., 2013). Discrepancies in the observed relationship between PRRSV prevalence in farm populations and that in wild populations between North America and Europe may be due to unknown factors influencing these dynamics or reflect

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[1] Defined as an inanimate object that can carry and spread disease and infectious agents. Also called passive vectors.

differences in farming practices and wild pig populations. In both the United States and Europe, the significant difference between the PRRSV prevalence rates in domestic and wild populations indicates inefficient PRRSV transmission between the two groups (Corn et al., 2009; Ruiz-Fons et al., 2008).

2.1.3 Clinical signs

The clinical effects of PRRS depends on the virulence of the virus, whether an individual or population was previously exposed, the age of those affected, the size of the population/herd, the presence of other disease-causing agents, and environmental conditions or management practices (Iowa State University, 2023).

The primary factors determining disease severity and prevalence include pig density, pig movements, air quality, health status, housing systems, quantity of the virus present and strain of the virus (Done et al., 1996). Typically, in breeding-age gilts, sows, and boars, infection results in symptoms such as anorexia, fever, lethargy, depression, respiratory distress, and vomiting, as well as cyanosis (ears, abdomen, and vulva) (Iowa State University, 2023). Mortality of sows and gilts typically ranges from 1-4%, but can be as high as 10% in rare severe cases (Zimmerman et al., 2019a, 2019b). Boars generally exhibit few clinical signs and mortality is infrequent; however, some studies have shown infection can reduce semen quality (Zimmerman et al., 2019b). In general, mortality in older pigs is uncommon unless complicated with secondary infections (Wensvoort, 1993).

Reproductive issues are typical on infected farms and include decrease in number of sows and gilts that conceive or farrow, increase in premature farrowings, late term abortions, increased likelihood of stillborn, mummified, and weak born pigs, delayed return to estrus, and high pre-weaning mortality (Collins et al., 1992; Iowa State University, 2023). Duration of observed reproductive effects vary with herd size, but usually last 2 to 3 months (Iowa State University, 2023). During the first phase of acute infection occurrence, 1-3% of litters may be lost in sows that are at 21-109 days of gestation (Zimmerman et al., 2019a), but can be as high as 10-50% in rare cases where PRRS is acute (Hopper et al., 1992; Loula, 1991; Zimmerman et al., 2019a). During the second phase, late-term reproductive failure may vary from 20-95% of pregnant females (Wensvoort, 1993; Zimmerman et al., 2019b). Typically, 1-2% of litters are born dead (Zimmerman et al., 2019b), but litter mortality can be as high as 100% (Zimmerman et al., 2019a). Pre-weaned pig mortality is also highly variable, with studies reporting from 25% mortality (Stevenson et al., 1994) to 60% mortality (Zimmerman et al., 2019a).

For young growing and finishing pigs, clinical signs often include fever, depression, lethargy, stunting and expiratory dyspnea due to systemic disease, and pneumonia (Iowa State University, 2023). Postweaning mortality is often markedly increased and depends on the virulence of the strain and presence of secondary infections (Iowa State University, 2023). Acute PRRSV infection can elevate mortality by 12-20% (Zimmerman et al., 2019a). Where PRRSV is endemic, nursery pigs (after maternal antibodies wane) or replacement gilts and sows often exhibit the greatest signs of disease (Zimmerman et al., 2019a).

Infection with PRRSV induces an immune response that results in protection against future infection that varies among individuals and appears to be the same for the different strains of PRRSV (i.e., PRRSV-1 and PRRSV-2) (Zimmerman et al., 2019a). Resistance to infection increases with age (Klinge et al., 2009).

2.2 Purpose and need for the proposed action

PRRS is the most economically important disease of domestic swine in North America, Europe, and Asia, costing producers in the United States more than $600 million annually (Holtkamp, 2013, Reiner et al., 2009). PRRSV is a member of the mammalian arterivirus group, which share pathogenic traits, including macrophage tropism[2] and the ability to cause serious disease and lasting infection. In young pigs, infection with PRRSV results in respiratory illness, including cough, fever, and lowered growth performance. In pregnant sows, PRRSV infection often results in reproductive failure, as well as chronically infected and low birth weight piglets. Commercial vaccines are available but have not been effective against the disease (Whitworth et al., 2016), and although genetic selection for natural tolerance is an option, success to date has been limited, possibly due to the genetic diversity of the virus (Boddicker et al., 2014). Perhaps not surprisingly, PRRSV has therefore become established in most swine-producing regions of the world, with only a few exceptions. It is generally assumed that 60-80% of domestic herds in pig-dense regions are typically infected (Boehringer Ingelheim, 2023a).

Thus, the purpose and need of the proposed action is to approve an IGA in domestic pigs (*S. scrofa*) that will be used to build populations of PRRSV-resistant pigs on farms to lessen the economic impact of the disease and to improve the health of domestic pigs in the United States.

2.3 Approach to risk assessment

Using principles outlined in CVM's Guidance for Industry (GFI) 187A and B, this risk analysis focuses on determining if there is any more environmental risk associated with the pigs with the IGA than its comparator, in this case domestic pigs without the IGA.

In a National Research Council (NRC) report titled, "Animal Biotechnology: Science Based Concerns" (NRC, 2002), the NRC describes risk [R] as the joint probability of exposure *[P(E)]*, and the conditional probability of harm given the exposure has occurred [*P(H|E)*], or R = *P(E)* x *P(H|E)*. Inherent in this equation is the concept that both exposure and harm (i.e., adverse effects) are required components of risk. The NRC provided four important definitions relevant to risk analysis of genetically engineered (GE)[3] organisms (NRC, 2002). These definitions are used herein and expanded to include other considerations relevant to this analysis. The risk is defined as the likelihood of harm resulting from exposure to the hazard. The hazard is defined as the GE organism itself and any act or phenomenon that has the potential to produce harm. Thus, in this assessment, the hazard is identified as the pigs that are heterozygous and homozygous for the IGA and their interactions with the ecosystem. The harm is defined as an adverse effect to the environment due to the hazard (i.e., pigs with the IGA). And exposure is defined as establishment and/or presence of pigs with the IGA in the environment. Presence alone, without genetic introgression and establishment, can result in environmental harm.

In order to determine if there would be any more risk to the environment from the approval of the IGA in pigs, the analysis considers how the IGA affects, if at all, the likelihood of exposure and the likelihood and severity of environmental harms of a domestic pig. This is determined by considering the phenotypic characteristics of the pigs with the IGA and determining any fitness advantages those characteristics may impart. The phenotypic characteristics and fitness of the pig with the IGA are then considered when evaluating whether the pigs with IGA pose any more environmental risk than domestic pigs without the IGA; that is, does the pig with IGA pose a

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[2] The specificity of a given virus for a cell type, tissue or species.

[3] CVM currently uses the term "animals with the intentional genomic alteration (IGA)."

greater likelihood of exposure [*P(E)*] and/or a greater likelihood and severity of harm [*P(H*|*E*)] than a pig without the IGA?

The likelihood of exposure *[P(E)]* is evaluated in Section 6.3 by considering the three main components driving the potential for exposure—likelihood of 1) escape, 2) survival and dispersal, and 3) reproduction and establishment. Because the conditions of approval will not require specific containment conditions for the pigs with the IGA (i.e., the pigs with the IGA can be reared at any facility and location with any level(s) of containment in the United States), this analysis assumes no specific conditions of confinement for production. Thus, the exposure of pigs with the IGA is unknown and can vary, similar to domestic pigs without the IGA.

Environmental harm of pigs with the IGA is characterized in Section 6.4 and the likelihood of harm is evaluated by considering the potential for the IGA to exacerbate documented and predicted environmental impacts of wild pig populations. This includes evaluating the potential for the IGA to alter phenotypic traits that could directly or indirectly impact the environment.

Given that escaped pigs containing the IGA could interbreed with conspecifics (i.e., wild pigs or escaped domestic pigs with or without the IGA), the exchange of genetic material could affect the likelihood and severity of harm to the environment. To determine this impact, the fate of IGA alleles and the potential for wild pig population growth was evaluated through simulation modeling. Only the homozygous state (IGA/IGA genotype) results in the PRRSV-resistant phenotype and thus, a potential fitness advantage to infected animals. Therefore, this EA places greater consideration on exposure to the environment from homozygous animals rather than heterozygous animals.

3. DESCRIPTION OF PRODUCT

3.1 Product definition and description

The product is defined as a “heritable intentional genomic alteration (deletion of exon 7 of *CD163* gene) in diploid, heterozygous and homozygous founder animals and offspring of domestic pigs (*Sus scrofa* domesticus). The deletion is intended to confer resistance to porcine reproductive and respiratory syndrome virus (PRRSV) in the homozygous animals known as PRRSV-Resistant Pigs.”

Post approval, semen, oocytes, live males, and live females from homozygous and heterozygous progeny with the IGA that descend from the initial progenitors will be sold. These animals are intended to create offspring with the IGA destined for slaughter and harvest. The conditions of approval will include the use on any farm in the United States with no specific requirements for containment and disposal of carcasses or manure. For the purposes of this document, the terms “pigs with IGA” refers to heterozygous and/or homozygous pigs and “PRRSV-Resistant pigs” refers to pigs homozygous for the IGA.

3.2 Production scheme

The intended alteration, resistance to PRRSV, would not be expected to result in changes to typical pig production procedures such as stocking density, breeding schedules, enclosure types, etc.

The resulting commercial pig intended for slaughter and harvest is a cross of multiple lines (purelines or breeds) of pigs. The paternal side, bred for traits such as growth efficiency and meat traits, traces directly back to a pureline, commonly referred to as a terminal line or a sire line. The maternal side, bred for reproductive efficiency, traces back to a cross between two purelines, commonly referred to as maternal lines or dam lines. Therefore, the maternal grandparents are of pureline origin. The maternal and terminal lines have been maintained in their original form and improved over generations through selective breeding.

The IGA was introduced into purelines during the creation of E0 animals. The E1 generation was heterozygous, and the homozygous state was first achieved in the E2 generation. After the E2 generations, the IGA has been maintained in the purelines via *in vivo* breeding techniques and producers will continue selective breeding to improve other desired traits.

The typical pork production scheme is shown in Figure 3-1. Within the diagram, red represents the portion of the pyramid that is owned and operated directly by PIC, a division of Genus; blue represents the portion of the pyramid that is operated by independent producers under contract with PIC for sale to other producers; and gray represents the portion of the pyramid owned and operated by customers for their internal use.

The top tier is the nucleus (representing fewer than 1% of all pigs in the pyramid) that maintains the purelines containing the IGA and continues selective breeding. For maternal lines, the nucleus will provide elite genetics in the form of semen, live males, and live females to enhance and grow the supply in the multiplication segment. For terminal lines, the nucleus will provide IGA genetics in the form of semen or live males for semen production to be used in the commercial tier.

The multiplication tier is a multigenerational population of maternal purelines and is devoted to making crossbred daughters containing the IGA for the commercial production tier; it also contains some pureline production of daughters to sustain themselves. The crossbred daughters containing the IGA will be distributed to the commercial tier to join previous generations of crossbred daughters. All commercial tier females will be crossed to terminal line males containing the IGA (born in the nucleus tier). The resulting pigs containing the IGA (slaughter pigs) are the primary commercial pigs harvested for pork products.

The breeding objective is to achieve a high percentage of homozygosity (IGA/IGA genotype) across all tiers of the pyramid. During the IGA dissemination ramp-up, heterozygous individuals will initially be produced in the multi-generational pyramid. As semen containing the IGA is distributed, and as females cascade down the production tier, zygosity of the IGA will move towards homozygosity across the entire pyramid. After homozygosity is reached, animals homozygous for the IGA will continue to be produced and disseminated through the pyramid to deliver improvements in other selective breeding program traits.

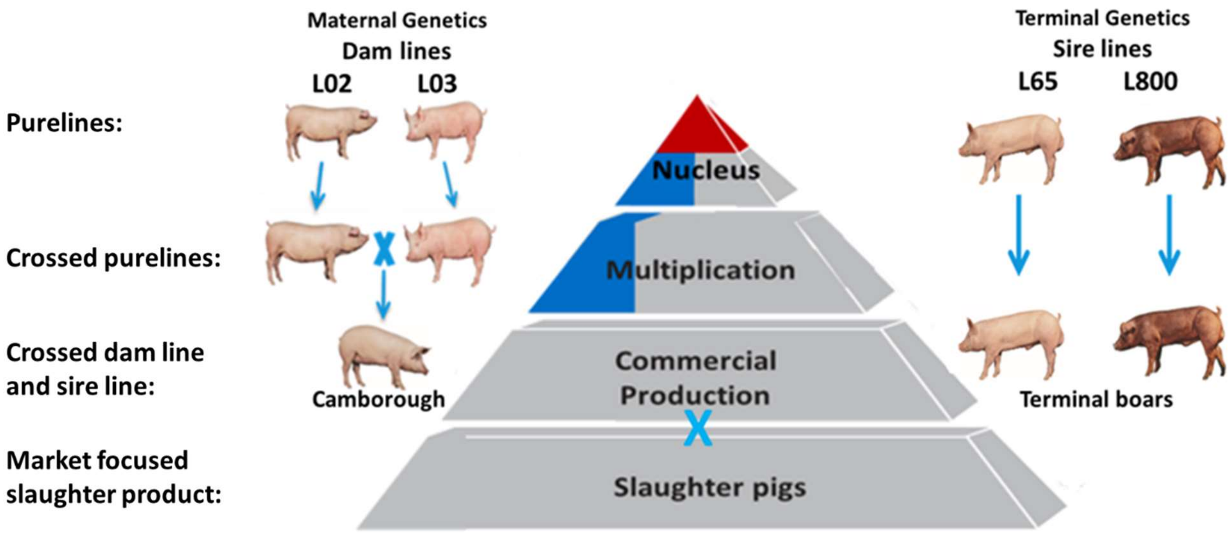

Figure 3-1. Commercial breeding strategy for PRRSV-resistant pigs.

3.3 Molecular characterization

The IGA is a deletion within the targeted region, exon 7, of the *CD163* gene in the genomes of elite maternal (e.g., PIC L02 and PIC L03) and terminal (e.g., PIC L65 and PIC L800) lineages of domestic pigs. The deletion is achieved by inducing two double strand breaks flanking the targeted region using a Cas9 protein-guide RNA complex. Exon 7 is the region of the gene responsible for coding the SRCR5 domain of CD163, the primary binding target for PRRSV. Therefore, if this region is deleted, infection is inhibited and resistance to PRRSV is conferred. The Cas9 complex was directly injected into fertilized porcine oocytes that were subsequently introduced into surrogate sows. No hazards regarding the IGA itself have been identified (e.g., use of foreign rDNA constructs, new expression products, use of antimicrobial resistance genes).

At birth, first generation (E0) piglets were screened for the presence of the IGA as well as any potential unintended edits to the genome (i.e., off-targets) using a combined strategy of 1) Illumina short read sequencing technology to query the targeted locus immediately around the CD163 locus; 2) Nanopore long-read sequencing technology to query a larger window around the targeted locus; and 3) hybridization-based sequence capture strategy to look at the entire coding region and adjacent regulatory regions of CD163 (Mark Cigan & Knap, 2022). The E0 founder animals were used as the IGA donor to create an E1 (second) generation by crossing founder E0 boars to wild-type gilts. The E1 progeny were screened for the presence of the IGA and a lack of any sequence variation at off-target loci. The advanced E1 animals were crossed (E1 x E1) to generate segregating E2 (third generation) progeny. The E2 generation was the first generation with the IGA in the homozygous state.

As expected, the results of the transcript analysis showed heterozygous animals had both edited and unedited transcripts, while the homozygous animals had only edited transcripts. Expression of CD613 protein assessed by western blots showed that the heterozygous animals exhibited a single band at approximately 150 kDa, and an additional band at approximately 130 kDa, corresponding to the protein from the edited allele (CD163-SRCR5Δ). Homozygous animals showed only the edited ~130 kDa band consistent with previous reports (Burkard et al., 2017). Details on the studies used to substantiate this conclusion can be found in the Freedom of Information (FOI) Summary that will be made available on AnimalDrugs@FDA.

3.4 Phenotypic characterization

The phenotypic differences between pigs that contain the IGA and domestic pigs that do not contain the IGA was evaluated by monitoring common parameters (i.e., weight, survival, rates of congenital defects, growth rate, rear leg structure, carcass composition, organ inspection) through early life, growing, finishing, and slaughter. A cohort of female pigs were monitored through their first litter and a cohort of male pigs were monitored through qualification as boars for stud. The animals monitored were E2 generation pigs of each genotype (pigs that were homozygous and heterozygous for the IGA and those who resulted from the same mating but without an IGA allele, i.e., homozygous null) and pigs from two genetic lines—one terminal genetic line and one maternal genetic line. Wildtype pigs from these lines (domestic pigs without the IGA that had not been part of the breeding to develop the E2 generation) were also compared to the pigs with the IGA. In addition, the animals were observed twice daily during the study and any adverse or unusual behavior was documented.

Starting at birth, pigs containing the IGA were similar for weight, survival, and rates of congenital defects (scrotal rupture, cryptorchid, and abdominal rupture). Through approximate market weight, pigs with the IGA were similar for growth rate, survival, front and rear leg structure, and carcass composition (as measured by ultrasound backfat and loin depth). A subset of the trial animals was selected for post-mortem organ inspection. Lungs, livers, hearts, and digestive tracts were normal for pigs containing the IGA. The remaining boars and gilts were grown to reproductive maturity. Boars containing the IGA were trained for semen production[4] at the same rate as boars that do not contain the IGA. Gilts containing the IGA conceived, gestated, and had litters with similar numbers of piglets compared to gilts that do not contain the IGA. Throughout the trials, survival and morbidity were similar across the zygosities (i.e., whether pigs possessing the IGA had one or two copies of the IGA). No differences in measured traits between pigs with the IGA and domestic pigs without the IGA were identified. In addition, no adverse or unusual behavior was documented. Details on the studies used to substantiate this conclusion can be found in the FOI Summary that will be made available on AnimalDrugs@FDA.

3.5 Genotypic and phenotypic durability

The desired IGA was inherited stably from the E0 to E1 and from the E1 to E2 generations of the pigs with the IGA. E1 generation pigs are offspring of E0 sires with the IGA mated to wild-type gilts. The E1 heterozygous pigs with no off-target alterations were crossed to each other within a given founder population to produce the segregating E2 generation consisting of homozygous, heterozygous, and homozygous null pigs. These results were consistent across the founder populations, thus demonstrating the stability of the desired IGA and establishing the genotypic durability of the IGA across three generations. Details on the studies used to substantiate this conclusion can be found in the FOI Summary that will be made available on AnimalDrugs@FDA.

The durability of the PRRSV-resistance phenotype in homozygous animals to representative Type I and Type II viral isolates across four generations of pigs (E0 to E3) was demonstrated

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[4] In order to collect semen for artificial insemination, a dummy sow is used for the boar to mount and simulate mating. To increase the success of collecting high quality semen, a boar is often slowly introduced to the dummy sow over a period of a few weeks.

utilizing live animal viral challenges and cell-based assays. The isolates used to test the phenotypic durability of the PRRSV-resistant pigs are representative of both Type I and Type II PRRSV and have been previously cited in literature focused on PRRSV challenges of pigs, including other gene-edited pigs (Ladinig et al., 2015; Wells et al., 2017; Whitworth et al., 2016; Yan et al., 2022).

Genus has developed genotypic and phenotypic durability plans for post-market record keeping ensuring that no unintended or undesired changes occur during the production of pigs that contain the IGA. Details on the durability plans can be found in the FOI Summary that will be made available on AnimalDrugs@FDA.

3.6 Effectiveness/claim validation

Homozygous, heterozygous, and null pigs from four generations (E0 to E3), as well as wild-type pigs, were challenged with multiple PRRSV Type I and II isolates. PRRSV resistance was demonstrated in homozygous pigs across all tested generations. PRRSV resistance was not conferred to heterozygous pigs. Details on the studies used to substantiate this conclusion can be found in the FOI Summary that will be made available on AnimalDrugs@FDA.

4. BACKGROUND ON DOMESTIC PIG PRODUCTION AND WILD PIG POPULATIONS IN THE UNITED STATES

4.1 Pig farming practices in the United States

4.1.1 Reproduction and breeding

Usually, male and female pigs attain puberty and physiological maturity between age 5 and 6 months (Reiland, 1978). Exposure to boars leads to heat (estrus, occurring every 21 days) in gilts and sows, which determines the ideal time for mating or artificial insemination (AI) to fertilize oocytes (Sterle & Safranski, 1997). Commercial operations now predominantly use AI for fertilization, which has facilitated global improvements in fertility, genetics, labor, and herd health (Knox, 2016).

The total gestation period from conception to birth of piglets (farrowing) on average is about 114 days, with a range from 111-120 days; gilts tend to have a shorter pregnancy. Farrowing results in a litter size of 8-12 piglets and the national average is 10.7 piglets per litter. Piglets feed on milk from lactating sows/gilts for 19-21 days before they are separated from the sows. The weaned piglets (13-15 lbs.) are moved to nursery rooms and fed on dry nursery feed pellets for 8-10 weeks to let them develop into growers/feeders (50-60 lbs.) and moved to the grower pens (National Pork Board, 2023). Grower/finishers are fed *ad libitum* until they attain the market weight of about 286 lbs. at around age 6 months. Based on the age of the pigs that are bought and sold, there are five types of commercial pig farms: farrow-to-finish farms, farrow-to-wean farms, wean-to-finish farms, farrow-to-nursery farms, and finishing farms (Farms.com, Ltd., 2023).

4.1.2 Distribution and types of pig farms in the United States

Based on the 2017 county-level livestock census data from the USDA's National Agricultural Statistics Service,[5] domestic pig farms are distributed throughout most of the United States, but their size varies considerably across regions (Figures 4-1a, 4-1b). Large farms (≥ 100 hogs) are

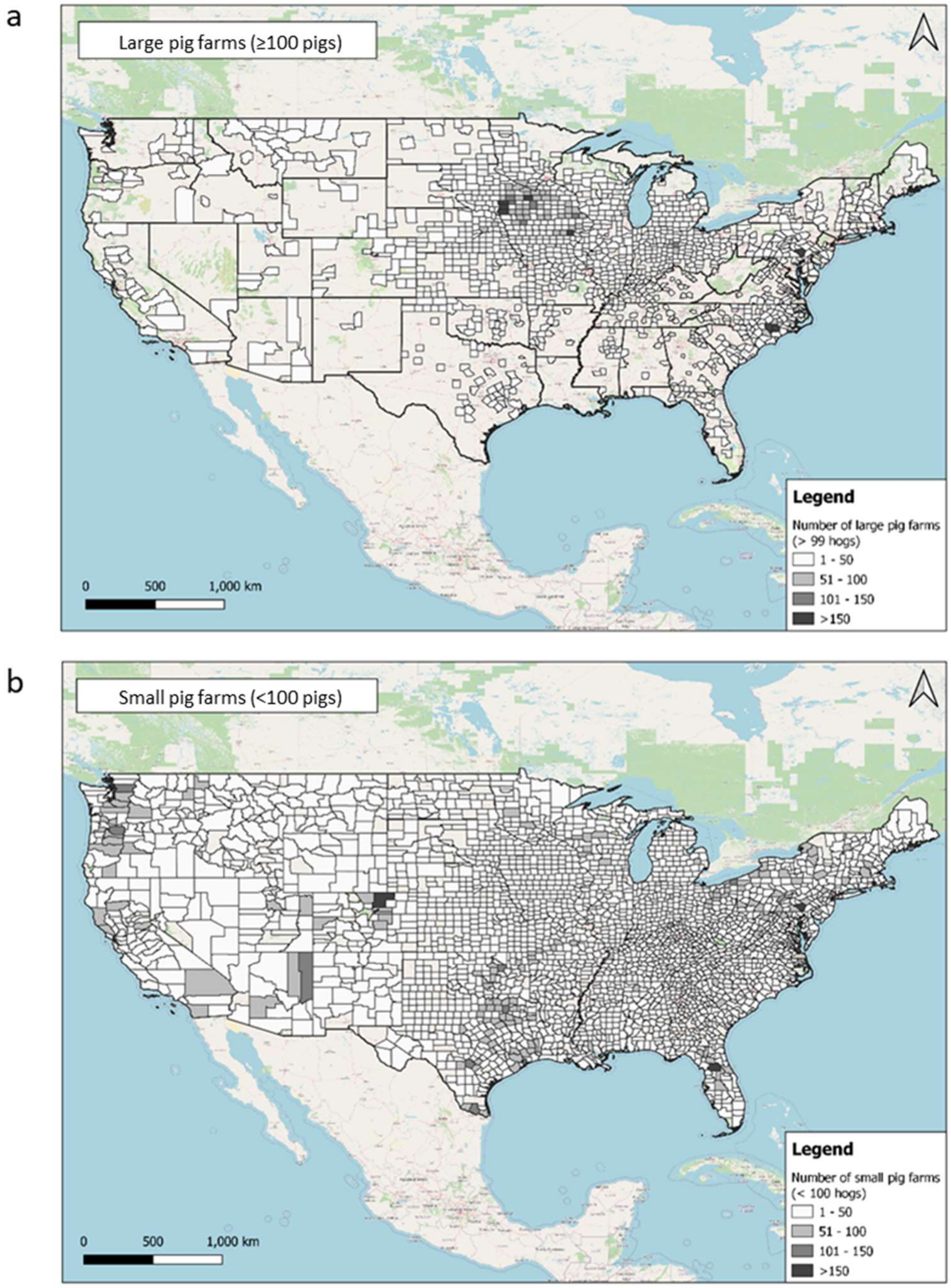

Figure 4-1. Total number of pig farms per county, for a) large (≥ 100 hogs) and b) small (< 100 hogs) farms; missing counties did not have census data.

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[5] https://quickstats.nass.usda.gov/results/E84832A6-A04B-3843-9124-65240129AB10; accessed May 11, 2022

found in approximately 41% of counties and the highest number of large farms are in Iowa, Minnesota, and North Carolina. In contrast, small farms (< 100 pigs) are found throughout the entire United States and exhibit greater variation in the number of farms per county.

Also according to the most recent Census of Agriculture conducted by USDA in 2017,[6] 99.3% pigs in the United States[7] are contained within 14,316 large-enterprise facilities (i.e., operations with ≥ 100 pigs).[8] The remaining 0.7% of the pig population in the United States is housed in 52,123 small-enterprise facilities (i.e., operations with < 100 pigs). The consolidation of swine into fewer, larger-scale industrial facilities is the result of a long-term trend, with the number of pig farms in the United States decreasing by more than 70% since 1990 (USDA, 2021b). Figure 4-2 illustrates this trend.

Based on a USDA survey conducted in 2012 (USDA, 2015), more than 75% of large-enterprise facilities hold weaned pigs only. Units on these large-enterprise farms are primarily grower/finisher units or wean-to-finish units. Less than 20% of large-enterprise operations include a breeding herd with gestation and farrowing. The survey of small-enterprise farms (USDA, 2014) found that smaller operations most commonly hold sows and gilts for breeding (58.1% of operations) and market pigs fed for slaughter (55.9% of operations), followed by boars and young males for breeding (42.9% of operations) and pigs not yet weaned (23.0%).

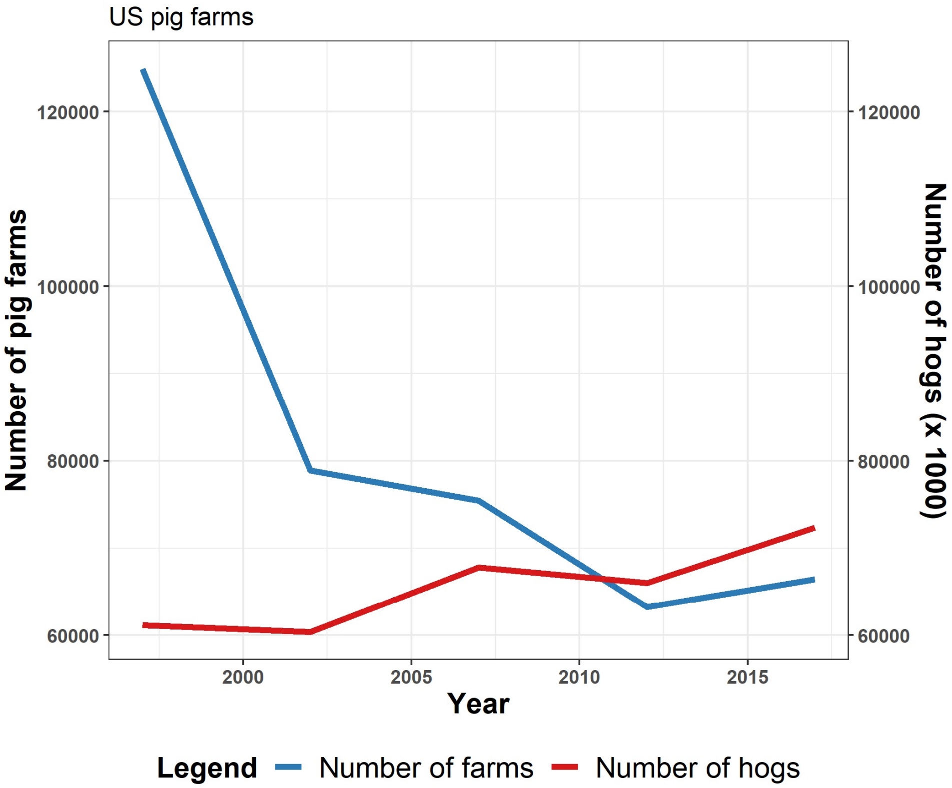

Figure 4-2. Number of pig farms and number of hogs in the United States.9

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[6] The results of the 2022 Census of Agriculture are expected to be released in 2024 (https://www.nass.usda.gov/AgCensus/FAQ/2022/index.php)

[7] USDA Quick Stats Database https://quickstats.nass.usda.gov/; accessed March 4, 2022

[8] Large-enterprise swine facilities as defined by USDA (2015).

[9] Source: https://quickstats.nass.usda.gov/results/92958049-5687-345E-848A-310A28254115, accessed March 4, 2022

4.1.3 Containment on pig farms

There are two types of containment on a farm: physical and procedural. Physical containment refers to the physical barriers (e.g., walls, pens, fences) that are present on a farm to prevent free movement of the pigs. Procedural containment refers to operating procedures in place to limit the risks introduced through exposure to people and other animals, including the likelihood of animal escape. Examples of procedural containment measures include security, limited access, emergency protocols, employee training, biosecurity plans, etc.

Physical containment

Although a range of conditions for physical confinement can be found throughout the United States, indoor production facilities are typically equipped with a minimum of two levels of physical containment—pens and buildings. For sites with outside access, one level of physical containment is typically in place. In addition, conditions of physical confinement vary greatly between small and large pig operations. Large operations typically have greater physical controls and reduced outside access for pigs (Table 4-1,
Table 4-2). For large operations, > 97% of pigs (and up to 99.8%) have no outside access, regardless of facility type (e.g., gestation housing, finisher housing) (Table 4-1) (USDA, 2015). In contrast, most small farm facilities (> 66.8%) allow some level of outside access (

Table 4-2) (USDA, 2014). Figure 4.3 provides a visual representation of the number of pigs held on different types of farms.

Table 4-1. Percentage of pigs by facility type and phase for large farms (≥ 100 pigs) (USDA, 2015). Values in parentheses are standard errors.

	Facility Type				
Access Type	**Gestation**	**Farrowing**	**Nursery**	**Grower / Finisher**	**Wean-to-finish**
Total confinement	76.7 (7.5)	88.6 (6.9)	97.1 (1.7)	85.8 (6.0)	87.4 (3.1)
Open building with natural ventilation and no outside access	20.7 (7.6)	10.3 (7.0)	2.7 (1.7)	13.7 (5.8)	11.4 (3.0)
Total with no outside access	**97.4**	**98.9**	**99.8**	**99.5**	**98.8**
Open building with outside access	2.0 (0.4)	0.6 (0.1)	0.2 (0.1)	0.4 (0.2)	1.1 (0.4)
Lot with hut or no building	0.4 (0.1)	0.2 (0.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Pasture with hut or no building	0.3 (0.1)	0.4 (0.2)	0.0 (0.0)	0.0 (0.0)	0.1 (0.0)
Total with outside access	**2.7**	**1.2**	**0.2**	**0.4**	**1.2**

Table 4-2. Percentage of small farms (< 100 pigs) containing weaned pigs and sows/gilts by facility type and phase (USDA, 2014). Values in parentheses are standard errors.

Access Type	Facility Type	
	Weaned	Sow/Gilt
Total confinement with mechanical ventilation	16.4 (2.4)	8.2 (1.8)
Open building with no outside access	16.8 (2.4)	15.0 (2.3)
Total with no outside access	**33.2**	**23.2**
Open building with outside access	41.0 (3.2)	40.3 (3.2)
Fenced lot with or without hut/shelter	16.9 (2.5)	23.6 (2.8)
Fenced pasture with or without hut/shelter	7.5 (1.7)	11.6 (2.1)
No facilities; pigs roam free with no fence	1.4 (0.8)	1.3 (0.7)
Total with outside access	**66.8**	**76.8**

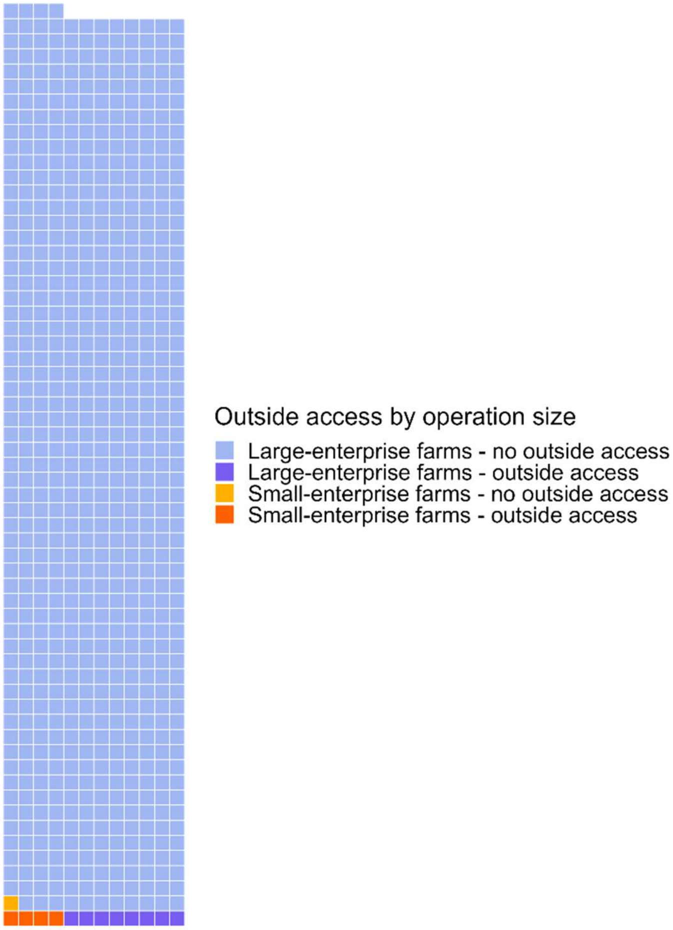


Figure 4-3. Estimated proportion of all domesticated pigs in the United States, with and without outside access, for small (<100 pigs) and large-enterprise (≥100 pigs) farms based on data from the 2017 USDA Census of Agriculture7 and USDA pig operation surveys (USDA, 2014, 2015). Each grid cell represents 100,000 pigs.

Procedural containment

Large farms are more likely to have greater procedural controls. For example, large farms have been shown to adopt measures to reduce disease transmission more frequently than small farms (Pudenz et al., 2019). The increased investment in biosecurity is associated with increased layers of physical containment (e.g., doors, fences, barriers preventing excavation under fences) and procedural controls (e.g., establishment of a perimeter buffer area, a line of separation, one entry point, restricted vehicle entrance) (Alarcón et al., 2021; Pudenz et al., 2019).

Biosecurity plans are developed to prevent the introduction of disease to the domesticated pigs from external sources (e.g., people, animals). Examples of containment measures utilized for biosecurity purposes include isolating pigs before introducing them to the herd, locating pigs at a minimum distance from other animals, and fencing the perimeter of the farm to prevent interaction with wild animals. In many cases, biosecurity practices may also overlap with general good husbandry and animal welfare practices. At many large farms, personnel are trained at the date of hire in relation to animal welfare and animal handling (and continued training occurs on the job). Some sites will also require employees to be Pork Quality Assurance (PQA+) certified. This is an educational program that represents the industry's commitment to continuous improvement of production practices. It addresses food safety, animal well-being, environmental stewardship, worker safety, public health, and community.

For sites where progenitors are raised (e.g., nucleus sites), access is extremely limited and some of the strictest biosecurity plans are implemented. For sites where only pre-pubertal progeny intended for meat production are housed, risks of a disease outbreak are lower (e.g., no risk for sexual transmission) and biosecurity plans are not as stringent. In general, biosecurity plans include authorization for visits by non-farm employees, designated down-time requirements for people moving site-to-site, health checks, controlled one-way animal flows (i.e., prevent lower-health animals compromising a higher-health site), shower-in-shower-out policies or use of disposable outer garments, disinfection of vehicles and equipment, rodent and pest control, and maintaining physical barrier integrity (e.g., fences, doors, bird netting, etc.).

With regard to farm facility protocols in the event of a natural disaster, USDA has published a general preparedness fact sheet for disaster livestock planning, including tips on preparing a livestock evacuation kit, making an evacuation plan, building secure shelters, and assessing farm safety (USDA, 2016). USDA has also published hurricane guidance specifically for hog farmers in the southeast (USDA, 2021a). Example guidance includes instruction on securing facilities to ensure animal containment during storm events (USDA, 2021a). With the exception of hurricane guidance, specific guidance for hog farmers in the event of other natural disasters is lacking, likely because their impact on hog farms is rare or not significant.

4.1.4 Disposal of pig carcasses and waste

Pig carcass and waste disposal procedures are expected to follow local regulations, which vary across the United States. For mortalities that occur on the farm, pig carcasses are typically composted or buried on-site, transported to a landfill, or sent to a third-party for rendering or incineration. Waste (e.g., urine, feces, blood) is typically stored on-site for application to nearby agricultural fields or sold to other farms for application to fields. Pigs intended for slaughter are transported from the farm to the slaughterhouse. These facilities are regulated by the USDA and the Environmental Protection Agency (EPA), including carcass and waste disposal.

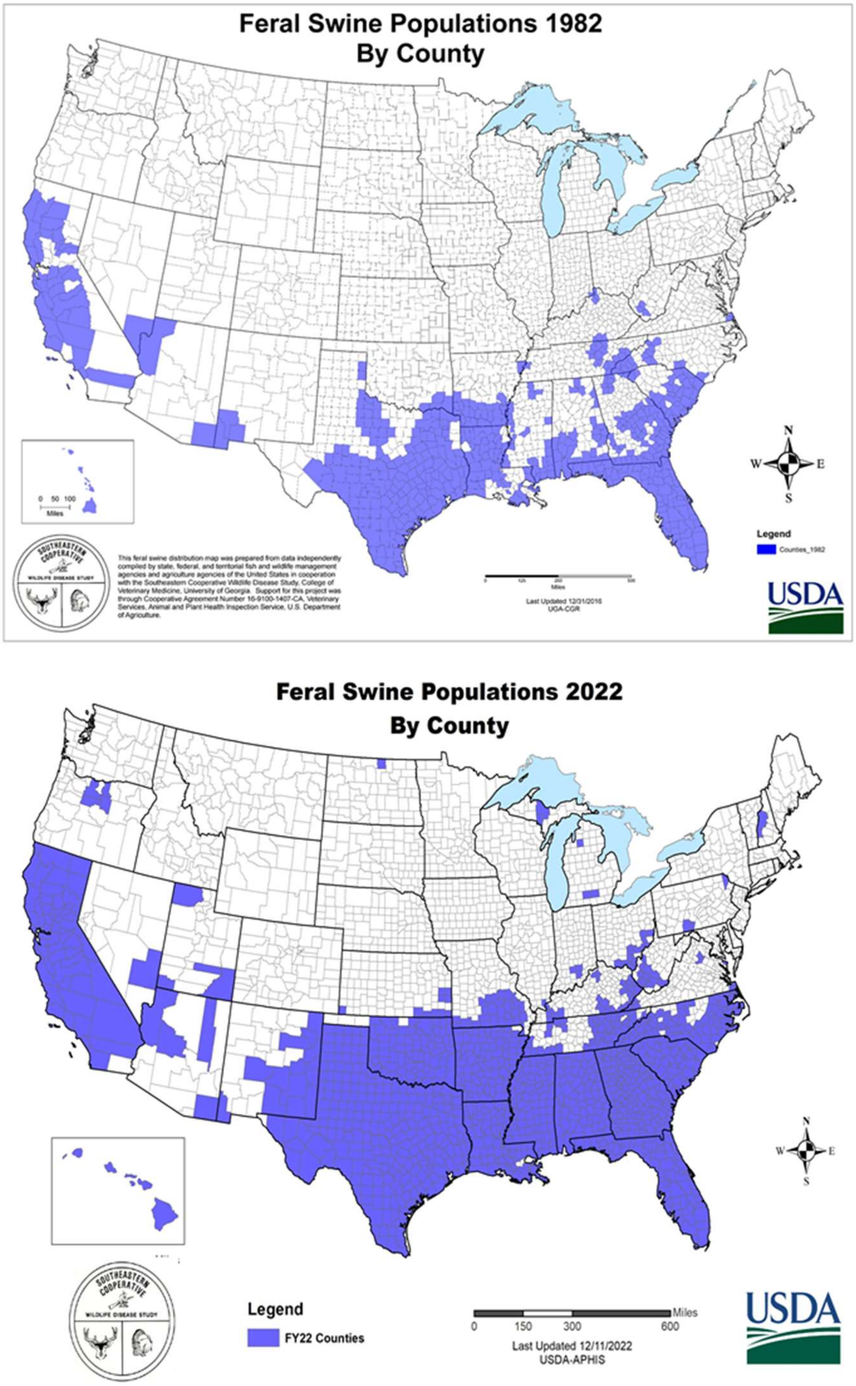
4.1.5 Transportation of domesticated pigs in the United States

The transportation of domesticated pigs in the United States is regulated by federal and state authorities, including the Department of Animal Health run by each state, the Department of Transportation and the USDA. Domestic pigs are typically transported in enclosed livestock trailers or by truck operated by a licensed, commercial carrier. The trailer is organized into multiple, configurable compartments to separate and keep the animals spread out and contained, as appropriate for their size and age.

4.2 Wild pigs in the United States

All domesticated pigs (*S. scrofa*) trace their ancestry back to Eurasian wild boar that were domesticated approximately 9,000 years ago in the Middle East and China (VerCauteren et al., 2019). Since that time, humans have facilitated the introduction and movement of pigs throughout the world. The first introduction of *S. scrofa* to North America is thought to have occurred during the late 15th century by Christopher Columbus (VerCauteren et al., 2019). Spanish explorers helped to continue the spread of pigs along expedition routes through the southeastern United States by intentionally releasing them into the wild to establish a sustainable food source (VerCauteren et al., 2019). Up until approximately the 1960s, domestic pigs were seasonally released into the wild so they could fatten up on mast crops (i.e., fruits and buds of forest trees and shrubs). The initial introduction, and subsequent and continued introductions that occurred into the 20th century, consisted of domesticated pigs of European wild boar ancestry. During the late 19th century, European wild boar were also translocated to the United States and intentionally released on private hunting preserves (Texas A&M AgriLife Extension, n.d.). Today, wild pig populations consist of a heterogenous mix of pure feral pigs (escaped/released domesticated Eurasian wild boar), Eurasian wild boar, and hybrids of the two lineages (Mayer & Brisbin, 2009; VerCauteren et al., 2019). Given that *S. scrofa* is not native to North America and the majority of wild pigs contain some ancestry from domestic pigs, the terms wild and feral pigs are used interchangeably in the literature and are considered synonymous for the purpose of this EA. However, the term “wild pig” will be used in this document when discussing wild or feral pig populations.

The range of wild pigs in North America has expanded rapidly over the last half century and is expected to continue expanding across most of the United States in the future (Figure 4-4) (Snow et al., 2017; USDA, 2023). Wild pig densities, however, vary considerably across the United States, and remain the highest in the southeast (Figure 4-5). The southeastern regions (i.e., between Texas and North Carolina) of the United States have the highest densities, whereas densities in northern regions and the western side of the Rocky Mountains remain relatively low. Spread does not appear to be completely hindered by environmental conditions (Snow et al., 2017) and it remains unclear whether management efforts will effectively halt or slow the spread of wild pigs to new counties and states.



This feral swine distribution map was prepared from data independently compiled by state, federal, and territorial fish and wildlife management agencies and agriculture agencies of the United States in cooperation with the Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia. Support for this project was through Cooperative Agreement Number 16-9100-1407-CA, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture.

Last Updated 12/31/2016
UGA-CGR

Last Updated 12/11/2022
USDA-APHIS

Figure 4-4. Change in distribution of wild pig populations from 1982-2022 (USDA, 2023).

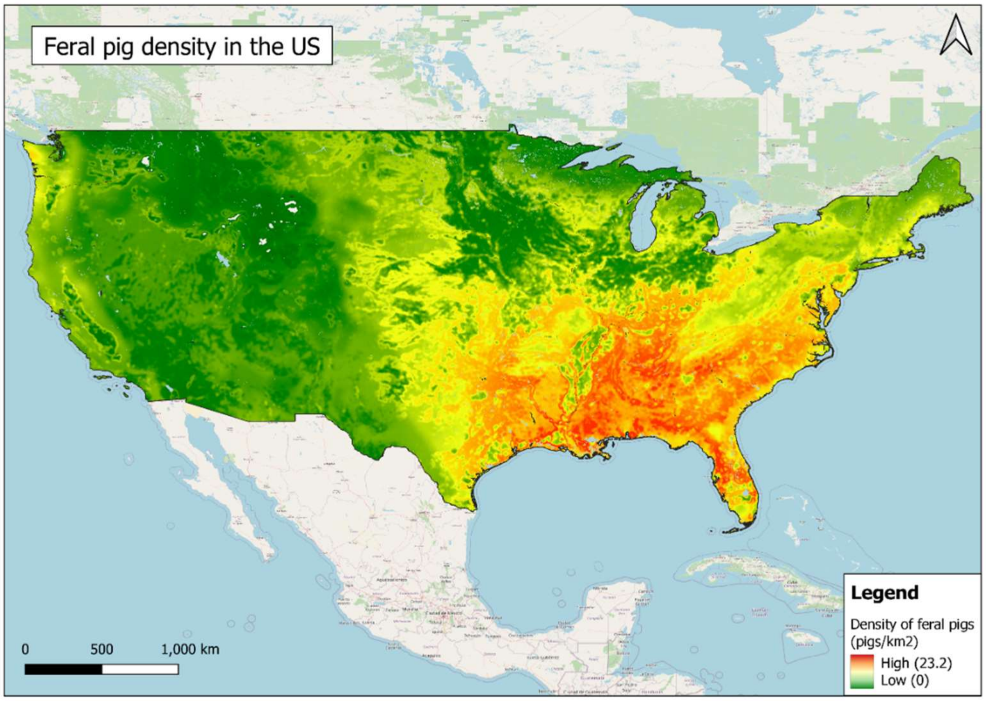


Figure 4-5. Map of predicted wild pig densities for 2021.[10]

The expansion of wild pigs in North America can be largely attributed to the presence of economic incentives that encourage private citizens to intentionally translocate wild pigs, legally and illegally, to new locations and habitats (VerCauteren et al., 2019). For example, range expansion in California has been facilitated by “a combination of multiple hunting-related introductions, deliberate releases of domestic pigs, and possibly increased forage availability associated with agriculture development” (Waithman et al., 1999). Similarly, the frequency of wild pig translocation has been found to increase with social interest and opportunity for recreational hunting of wild pigs (Tabak et al., 2017). Holding facilities—enclosures “where wild pigs are temporarily held before being sold for slaughter or released into captive hunting facilities” (Mangan et al., 2021)—specifically have been implicated as foci for frequent translocations. Genetic analysis of wild pig populations further indicate that the frequency of long-distance translocation events is most strongly related to recreational pig hunting (Hernández et al., 2018; Tabak et al., 2017). However, these operations are becoming increasingly regulated or banned and so may, over time, contribute less to the spread of wild pigs than they did historically.[11,12,13]

Mayer and Brisbin (2009) summarized reports of wild pig populations in the United States (Table 1 in Mayer & Brisbin, 2009). Populations characterized as “recent releases/escapees” are estimated to be between 0 and 100 pigs based on a “rough bounding estimate.” These

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[10] Data source: https://data-usfs.hub.arcgis.com/documents/usfs::terrestrial-condition-assessment-tca-feral-pig-density-map-service/explore; accessed August 31, 2023 (last updated August 22, 2022).

[11] https://www.democratandchronicle.com/story/sports/columnist/roth/2014/05/18/hog-heaven-hunters/9227217/; accessed August 31, 2023.

[12] https://ediblemarinandwinecountry.ediblecommunities.com/shop/free-ranging-pigs-not-so-free-taking; accessed August 31, 2023.

[13] https://trackbill.com/bill/california-senate-bill-856-wild-pigs-validations/2199129/; accessed August 31, 2023.

populations largely result from sport hunting sources, including illegal releases and escapes from fenced preserves, rather than from farm escapes (Mayer & Brisbin, 2009).

5. AFFECTED ENVIRONMENT

The intended use of pigs that contain the IGA is as a source of food products for human consumption and as other biological products, such as animal feed. As such, the IGA will be sold to pork-producing facilities within the United States in the form of pre-pubertal pigs, mature pigs, oocytes, or sperm that contain the IGA. Pork production facilities will use these products that contain the IGA to create progenitors to produce offspring that contain the IGA destined for meat consumption. This EA does not focus on specific facilities or locations within the United States because specific conditions of confinement are not proposed. Therefore, the affected environment includes the entire United States and its territories.

The United States has a total area of around 3,800,000 square miles comprising of 48 contiguous states and the District of Columbia, plus Alaska and Hawaii (CIA, n.d.). The mainland of the United States is bordered by the Atlantic Ocean on the east coast, the Pacific Ocean on the west coast, the Gulf of Mexico on part of the southern coast, along with Mexico, and Canada to the north. Alaska is located at the northwestern end of North America. It borders the Pacific Ocean to the south and southwest, the Bering Strait to the west, and the Arctic Ocean to the north. Hawaii is located southwest of the mainland, in the Pacific Ocean. The landscape of the United States is quite diverse and encompasses desert, mountains, forests, plains, tundra, beaches, wetlands, estuaries, freshwater streams, lakes, etc. (Gopnik, n.d.). It has uninhabited remote wilderness areas, sparsely populated rural towns, suburban areas and densely populated cities. The highest elevation is 6,190 m (Denali) and the lowest is -86 m (Death Valley).

The climate of the United States varies depending on latitude and geologic formations (e.g., mountains, oceans). It can range from semi-arid in the southwest to subtropical in Florida to arctic in Alaska (Gopnik, n.d.). Temperatures show a strong gradient across regions and seasons for much of the United States. The daily mean temperature ranges from 0°C in January to 23.6°C in July. Highs of over 37.8°C are routinely measured in southern coastal states in the summer. Lows of -6°C are often recorded in Alaska in the winter (World Bank Group, n.d.).

The amount and timing of precipitation also varies across the country and by season. Amounts range from more than 180 inches per year along the Pacific northwest to less than 5 inches in the west and southwest (World Bank Group, n.d.). Peak rainfall season of the Great Plains and midwest states is in late-spring while west coast states have a distinct rainy season during winter. Many Gulf and Atlantic coastal regions experience summertime peaks. Heavy snow is common in the northern states and Alaska during winter (Gopnik, n.d.).

The United States is subject to almost every kind of severe weather, including thunderstorms, snowstorms, flooding, tornadoes, and hurricanes (CIA, n.d.). Earthquakes occur in Alaska and on the west coast due to the presence of heavy tectonic and volcanic activity. There are also several active volcanoes on the Hawaiian Islands. Forest fires can occur in hot, dry climates in the southwest, especially in times of drought (CIA, n.d.).

6. RISK CHARACTERIZATION

Risk of harm to the environment from the approval of the IGA is assessed in this section by evaluating whether the pigs with the IGA pose any more environmental risk than domestic pigs without the IGA. That is, does the pig with the IGA pose a greater likelihood of exposure [*P(E)*] and/or a likelihood and severity of harm [*P(H|E)*] than a pig without the IGA?

To assess this question, it is critical to understand whether the IGA affects the potential for environmental exposure and harm of a domestic pig. This is determined by considering the phenotypic characteristics of the pigs with the IGA and any fitness advantages those characteristics may impart. For example, the IGA is known to confer resistance to PRRSV in homozygous individuals; thus, if escaped pigs with the IGA reproduce with wild pigs, the IGA could impart a fitness advantage to wild pig populations by increasing the number of individuals who are resistant to PRRSV and improving survival and reproduction in wild pig populations. This could potentially result in greater invasiveness and environmental harm of wild pigs. Alternatively, if the IGA imparts little to no fitness advantage, the exposure and environmental harms posed by pigs with the IGA would be the same as domestic pigs without the IGA. The potential fitness advantage of the IGA is evaluated in Section 6.1 below.

The information on fitness is used in Section 6.2 to determine whether environmental exposure (i.e., establishment and/or presence) of pigs with the IGA is more likely than pigs without the IGA by examining the likelihood of escape, survival, dispersal, reproduction and establishment. The environmental harms caused by wild pig populations are identified in Section 6.3, and the potential for pigs with the IGA to cause greater environmental harm than pigs without the IGA is also evaluated. In addition, simulations were performed to further evaluate the fate of the PRRSV-resistant phenotype and the potential for the IGA to increase the growth of wild pig populations, and thus the environmental harm, in the event the IGA enters wild populations. The population modeling is described in detail in Appendix A and the results are discussed in Section 6.4. The potential toxicity related to the IGA itself was evaluated in Section 6.5. Finally, conclusions on the overall risk of pigs with the IGA is presented in Section 6.6.

6.1 Evaluation of the fitness of pigs with the IGA

The IGA is intended to confer resistance to PRRSV in pigs homozygous for the IGA (IGA/IGA genotype). Pigs heterozygous for the IGA would not be resistant to PRRSV and would be no different from domestic pigs without the IGA. There are two ways the IGA/IGA genotype could alter the fitness (i.e., survival and reproductive success) of pigs in the wild: 1) by altering other (non-target) phenotypic traits, and 2) by providing resistance to PRRSV, the intended phenotypic change of the IGA. The IGA is not expected to alter other non-target phenotypic traits that could affect the likelihood of escape, survival, dispersal, reproduction and establishment (e.g., mating behavior, aggression). However, increased resistance to PRRSV could impart greater survival and reproductive fitness to the animal in the wild when exposed and infected with PRRSV. There are a few reasons why this fitness advantage is expected to be minimal.

First, any increase in survival or reproduction conferred by the IGA will depend on PRRS-mediated morality and reproductive failure in the wild. While the effects of PRRSV have been well documented in domestic pig populations (Morrow & Roberts, 1999), the effects of PRRSV in wild populations remain unknown. It is possible that PRRSV is less virulent in the wild than on farms, where conditions differ dramatically. For example, pigs on farms live in much higher densities than in the wild, facilitating the spread of diseases like PRRS. Relative to wild pigs, the immune system of domestic pigs may be more stressed due to greater exposure to other infections and the use of antibiotics. Also, it has been speculated that over time (decades to hundreds of years) wild populations may have evolved some natural level of resistance to PRRSV (ter Beek, 2018; Wu et al., 2011), although this has yet to be demonstrated empirically. Variation in antibody response to PRRSV, however, is known to have a genetic basis and selection on some of these quantitative trait loci could improve reproductive performance of PRRSV-infected pigs (Sanglard et al., 2020). Together, these factors, as well as the lower density of pigs in wild populations, may explain why when PRRSV enters wild populations, its subsequent spread is rather limited (Albina, 2000).

In contrast to the high incidence of PRRSV observed on farms, ranging from 25-35% (Morrison et al., 2015), the prevalence of PRRSV in the wild remains extremely low in North America, with regional studies estimating prevalence anywhere from 0-3.4% (Baroch et al., 2015; Corn et al., 2009; Pedersen et al., 2018) (Table 6-1). Prevalence is similar between males and females (Wyckoff et al., 2009). The low prevalence and limited spread of PRRSV in wild populations indicates that PRRSV is not a factor limiting the population growth of wild pig populations in North America.

Table 6-1. Estimated prevalence of PRRSV in wild pig populations in the United States

Spatial Sampling	Number of pigs surveyed	Highest Prevalence of PRRSV Reported	Years Sampled	Source
15 states (mostly southern)	162	2.5%	2011, 2012	Baroch et al., 2015
South Carolina	49	0%	2006, 2007	Corn et al., 2009
North Carolina	120	0.8%	2006, 2007	Corn et al., 2009
Hawaii	297	3.4%	2013-2015	Pedersen et al., 2018
Oklahoma	117	1.7%	1996	Saliki et al., 1998
Texas	117	1.7%	2004-2006	Wyckoff et al., 2009

Second, the genetic dominance of the IGA is homozygous recessive; therefore, resistance to PRRSV is only conferred in homozygous individuals. As a result, it would be difficult to establish a population of PRRSV-Resistant pigs in the wild. There are a few scenarios that could result in this occurring. The worst-case scenario would be a breeding pair of pigs homozygous for the IGA escaping and breeding together (F0). This would result in 100% of F1 offspring being homozygous for the IGA, and resistant to PRRSV. If these animals continued to crossbreed, a homozygous population could be established. This scenario is unlikely to occur because breeding pairs are rarely held on the same farm and intact boars are not commonly held on any farms (see Section 4.1.2). More likely, a pig with the IGA could escape and breed with a wild pig. Again, the worst-case scenario would be the escape of an animal homozygous for the IGA. Through Mendelian inheritance, the resulting F1 offspring would be 100% heterozygous for the IGA and not resistant to PRSSV. In order to produce homozygous F2 offspring, these F1 pigs would have to breed together, or backcross and breed with one of the homozygous F0 pigs (see

Figure 6-1). Because there is no selective advantage to being resistant to PRRSV, as discussed above, if the pigs with the IGA escaped into an area with wild pigs, they would not be expected to breed with other pigs with the IGA any more frequently than with wild pigs.

In theory, any actualized fitness advantage conferred by the IGA could result in negative feedback between the frequency of resistant individuals and the prevalence of PRRSV in the population. An increase in resistant individuals would be expected to lower the prevalence of PRRSV and, in turn, reduce the fitness advantage of being resistant. Epidemiological models of farm populations suggest that increasing immunity may help reduce the prevalence of PRRSV (Phoo-ngurn et al., 2019). Thus, any potential fitness advantage conferred by the IGA, if at all, would likely vary over space, but remain extremely low.

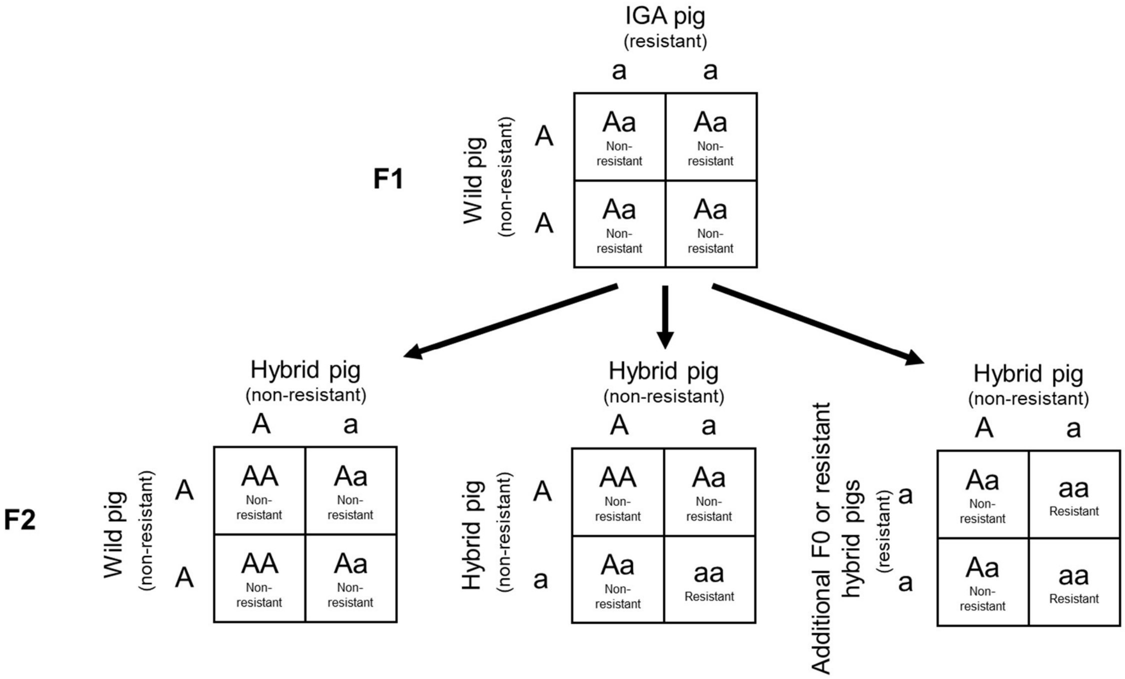


Figure 6-1. **Punnett squares showing the expected Mendelian inheritance of IGA in F1 and F2 offspring that would be produced from escaped IGA females**

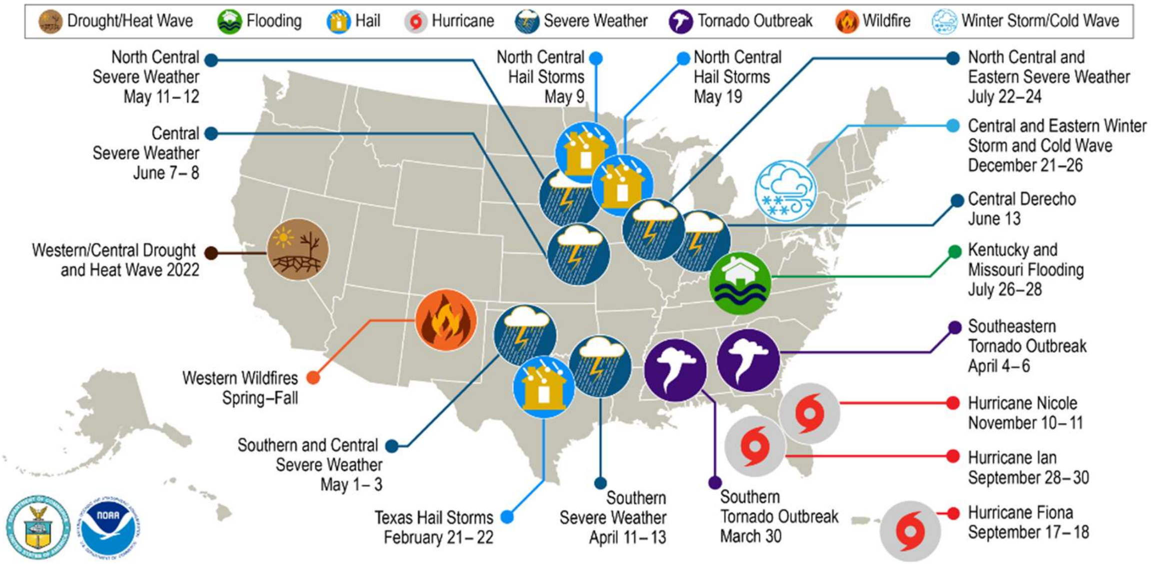
6.2 Likelihood of exposure

6.2.1 Escape

Domestic pigs without the IGA

The NRC categorized the likelihood of pigs escaping captivity as “moderate,” based on their ability to evade physical confinement measures (NRC, 2002). Several factors impact the ability of pigs to escape including, but not limited to, the levels of physical containment present on the farm, procedural containment measures in place, and frequency and severity of natural disasters. For pigs kept in facilities with outside access, pigs may escape their enclosures by using their ability to dig underneath, jump over, or move through holes in fencing or barriers (Coffin, 2018; Eastwood & Smith, 2020; Lineen et al., 2015; Pietrosemoli & Tang, 2020). The ability for pigs to evade physical barriers on a regular basis is largely mitigated in facilities with no outside access. Given that most pigs are kept indoors without access to the outside (Tables 4-1 and 4-2), the majority of pigs in the United States have a very low likelihood of escaping confinement, whereas the small percentage of pigs that have access to the outside would present a low to moderate likelihood of escape.

The possible impact of natural disasters on the likelihood of escape must also be considered. Natural disasters typically result in a total loss of physical containment leading to large escape or mortality events. The impacts of large-scale natural disasters are temporally and geographically varied (Figure 6.2). Common natural disasters in the United States include fire, tornadoes, hurricanes, flooding, and snowstorms. In recent decades (1980-2022), the United States has experienced an average of 7.9 weather events per year that are severe enough to cause more than one billion dollars in losses (NOAA, 2023). In areas where natural disasters are more frequent, strategies have been implemented to avoid adverse impacts to agricultural operations. For example, year-round hurricane preparation strategies have been implemented in North Carolina to prevent costly losses, including using hurricane forecast tracking to decide when to move animals to higher ground or to markets early (North Carolina Pork Council, 2020b). The North Carolina Swine Floodplain Buyout Program was established with the goal of buying and closing all pig farms in the 100-year floodplain (North Carolina Pork Council, 2020a, 2020b).



This map denotes the approximate location for each of the ***18 separate billion-dollar weather and climate disasters that impacted the United States in 2022.***

Figure 6-2. U.S. 2022 Billion-Dollar Weather and Climate Disasters. Reproduced from NOAA (2023).

In addition to escaping from farms, pigs can escape during transportation. For example, pig escape may occur during the loading/unloading process, in-transit, or following an accident. To improve effectiveness of movement of pigs, many best practices have been developed for pig handling (Garcia & McGlone, 2015; Global Ag Media, 2012; Grandin, 2003; National Pork Board, 2019; Outdoor Pig Farming, n.d.). For example, training staff on pig behavior, reducing noise and other distractions, using a pigs' natural tendency to move towards darker places, securing the gates and raceway and using ramps that are high enough to prevent pigs from jumping off, can all be employed to help to ensure a high standard of animal welfare and reduce the likelihood of escape during transportation. Pig escapes following trailer accidents have been reported in popular media articles, but tend to result in pig mortality, serious injury and/or recapture, rather than introduction to the wild (Associated Press, 2023; Daniels, 2019; Hooper, 2020; Lofaso, 2023; Schweigert, 2022). Therefore, like pig escapes from farms, pig escapes during transport are expected to be few and infrequent.

Pigs with the IGA

The pigs with the IGA could be present on any farm in the United States, and there will be no specific containment requirements for those farms producing or rearing pigs with the IGA. However, the IGA does not result in any changes to the phenotypic traits in the pigs that would cause the pigs to be more capable of escape than a pig without the IGA (e.g., they are not more aggressive, they are not more apt to dig). Therefore, the likelihood of escape of pigs with the IGA is the same as pigs without the IGA.

6.2.2 Survival and dispersal

Domestic pigs without the IGA and wild pigs

Factors affecting the survival and dispersal of pigs in the wild remain poorly understood, particularly for North American populations (VerCauteren et al., 2019). Survival and dispersal are expected to depend on multiple factors including, but not limited to, the age of the escaped pigs, natural factors (e.g., climate, predators, resources), and anthropogenic factors (i.e., hunting).

From the roughly half-dozen studies of North American populations, survival rates vary within age class and by population sampled, but do not significantly differ among age classes or from European wild pig populations (Miller et al., 2017; VerCauteren et al., 2019). The average survival rate in North America for all age classes is 0.56 (standard deviation [sd] = 0.26) with a typical lifespan of 8 years, and a maximum of 10 years (Henry & Conley, 1978; VerCauteren et al., 2019). Survivorship of neonate wild pigs in North America is currently unknown due to a lack of known-fate studies.

Although cause-specific mortality in wild populations remains understudied, “[a]nthropogenic mortality from recreational hunting or population control is thought to be the greatest source of mortality among wild pigs and wild boar” (VerCauteren et al., 2019). A similar pattern is observed in Central Europe, where hunting is a major factor driving mortality in wild populations (Keuling et al., 2013). In addition to humans, there are many natural predators of wild pigs in North America, including alligators, mountain lions, bears, coyotes, and many large raptors (Feral Hogs Extension, 2019). Predation is often opportunistic, however, dependent on prey density, and expected to have an overall minor impact on wild pig populations under most circumstances (Feral Hogs Extension, 2019). Mortality may also vary seasonally, with higher mortality in the wet season (Hayes et al., 2009) and post-farrowing periods (Henry & Conley, 1978). Although to a much lesser degree than hunting, resource availability and climate are expected to impact survival. However, due to a lack of studies, natural sources of mortality in North America are not well understood. Many pig traits are highly plastic, meaning that they change depending on environmental conditions; it is well known and widely documented that cranial morphology and hair density and thickness undergo relatively rapid changes as pigs transition from living on a farm to living in the wild (Bevins et al., 2014). Variation in some phenotypic traits (e.g., coat length, density) may also contribute to increased or decreased survival, but again these effects have not been evaluated in detail. Similarly, disease could affect survival, but remains understudied in wild populations and is not considered to be a primary factor influencing survival.

Dispersal distance and home ranges of wild pigs depend on many factors, including but not limited to, season, sex, resource availability, environmental conditions (e.g., weather, climate), vegetation type, and age. Males generally have greater dispersal distances (Keuling et al., 2010; Prévot & Licoppe, 2013) and home ranges (Kay et al., 2017) than females. Estimates of overall home ranges vary by method, with estimated averages ranging from 6.1 to 12.4 km^2 (Kay et al., 2017). In general, dispersal decreases (non-linearly) as pig density increases, with the effects of pig density on dispersal greatly diminishing when pig density rises above approximately 9 pigs/km^2 (VerCauteren et al., 2019).

Both domestic and wild pigs have also been shown to have high site fidelity, meaning that the pigs that do escape generally do not move far from their home ranges (Lopez et al., 2014; Mayer & Brisbin, 2009; Oliveira-Santos et al., 2016). Population genetic analysis has shown that wild pig populations exhibit “low rates of natural dispersal among proximate populations” (Tabak et al., 2017), and females from Europe and North America have been shown to exhibit a high degree of site fidelity (Podgórski et al., 2014) indicating that escaped female pigs are not expected to disperse far from where they escape. Domesticated pigs have also been shown to have a greater affinity for agricultural lands and be more accustomed to humans, often making them easier to capture or dispatch (Lewis, 1966). A recent pig escape from a Vermont farm illustrates this effect. About 250 pigs escaped from a fenced enclosure that had been damaged by vandalism (Associated Press, 2019). Within a month, almost all pigs (about 99%) had returned, credited in part to the farmer using food to lead the escaped pigs back to the farm. Therefore, it is expected that most escaped domestic pigs will be recaptured quickly after escape due to site fidelity and familiarity with human handlers.

<u>Pigs with the IGA</u>

The IGA does not result in phenotypic traits that would cause the pigs to disperse at a higher rate, faster, or further than pigs without the IGA (e.g., greater endurance). Similar to pigs without the IGA, it is expected that pigs with the IGA will remain close to the facilities due to site fidelity and familiarity with their human handlers and could be easily and quickly recaptured. Thus, the likelihood of dispersal of escaped pigs with the IGA is the same as pigs without the IGA.

The only fitness advantage that the IGA imparts on the pigs is the resistance to PRRSV. As described in Section 6.2, resistance only occurs for the pigs that are homozygous for the IGA. Resistance to PRRSV could in theory provide a fitness advantage for escaped pigs with the IGA and their offspring that are homozygous for the IGA by improving survival when infected with PRRSV compared to domestic pigs without the IGA. However, if there is no PRRSV in the wild population (prevalence is zero), there is no benefit for possessing the IGA and no fitness advantage. As discussed in Section 6.2, the prevalence of PRRSV in the wild remains extremely low in North America, with regional studies estimating prevalence anywhere from 0-3.4% (Baroch et al., 2015; Corn et al., 2009; Pedersen et al., 2018) (Table 6-1). Therefore, under natural conditions, the IGA is expected to confer little to no fitness advantage that results in improved survival in wild pig populations. Thus, the likelihood of survival of pigs with the IGA is expected to be the same as that for pigs without the IGA. This outcome is likely to hold true under a broad range of conditions. This conclusion is corroborated by the results of the population modeling contained in Appendix A and summarized in Section 6.4 below.

6.2.3 Reproduction and establishment

Domestic pigs without the IGA and wild pigs

Reproduction and establishment in the wild of an escaped domestic pig depends on the sex of the animal, environmental conditions where the escape occurred, density of feral pig populations and the phenotypic traits of the pig (e.g., coat length/color, cranial morphology).

The sex of escaped domestic pigs is an important factor determining the likelihood of reproduction, in that it largely determines whether reproduction is even possible. In North America, male piglets are typically castrated soon after birth (Harper, 2008; Rault et al., 2011; Spooner et al., 2014). There are two primary reasons that most male pigs are castrated: 1) to prevent poor boar meat quality, and 2) to prevent difficult behavioral problems (EFSA, 2004; Harper, 2008; McGlone et al., 1993). Castration improves boar meat quality by preventing boar taint, an unpleasant taste or odor associated with meat from non-castrated boars. Castration also causes observable behavioral effects, reducing aggressive behaviors including fighting and mounting (EFSA, 2004; von Borell et al., 2009). Increased feeding has been observed in castrated boars and is associated with a reduction in mounting behavior (EFSA, 2004).

In the United States, most females are artificially inseminated (Knox, 2016; USDA, 2015). This is especially the case on large farms. As a result, only select few males are used for breeding (Harper, 2008). For small farms, it is more common, with 42.9% of operations holding intact boars and young males for breeding (USDA, 2014). Where breeding males are held on small farms, there is an average of 5.5 breeding females per breeding male (USDA, 2014). For large farms that have breeding boars, sows outnumber boars significantly, with the ratio dependent on the method of mating. When using pen-mating, the boar-to-sow ratio ranges from 1:15 to 1:25 (Knox, 2016; Roese & Taylor, 2006). Using artificial insemination increases the boar-to-sow ratio range to 1:150-1:400 (Knox, 2016). Therefore, the likelihood of a breeding pair of pigs escape together is higher on a small farm than a large farm.

Multiple environmental factors are known to affect the ability of pigs to reproduce and establish in the wild. These include resource (food) availability, climate, and the presence of natural predators (including humans). The most important of these factors is thought to be resource availability, followed by climate (R. Miller, personal communication, 2022). The relationship between food availability and wild pig fecundity is well-established (VerCauteren et al., 2019). For example, reproductive success and litter size have been linked to mast production,[14] where the higher the mast production, the higher the litter size (Johnson et al., 1982; Matschke, 1967; VerCauteren et al., 2019). Climatic conditions can also mediate productivity by driving changes in the availability of forage resources, for example, via changes in precipitation patterns (VerCauteren et al., 2019). The impacts of predation and hunting on reproduction and establishment would primarily occur through increased survival, as discussed above.

In addition, reproduction is dependent on the available mates in the affected environment. This includes wild pig populations, as well as other domestic pigs. The location and density of wild pig populations in the affected environment would be the most likely to impact reproduction. It would be very unlikely for a mating pair (boar and sow/gilt) of domestic pigs without the IGA to escape at the same time. However, if a domestic pig escaped into an area with a high density of wild pigs in close proximity, the escaped animal would be more likely to locate a potential mate.

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[14] Fruits and buds of forest trees and shrubs on which wild pigs feed.

If the pig escaped into an area with a low wild pig density, or an area further from wild pig populations, it would be less likely to reproduce.

Phenotypic traits may also play a role in the reproductive potential of escaped pigs. Several traits developed through millennia of domestic breeding could result in an increased likelihood for reproduction and establishment in the wild. For example, there is some evidence, although limited, that introgression between wild and domesticated pigs has increased litter size of wild populations in Europe and North America (from 4.75-5.3) (VerCauteren et al., 2019); however, given the low heritability and polygenetic nature of reproductive traits, introgression between domestic and wild pig populations is not expected to result in a rapid increase of wild populations (Zak et al., 2017). To what degree traits involved in reproduction change as escaped pigs transition to conditions of living in the wild is not well understood. It is reasonable to expect that traits involved in reproduction, such as litter size, may also undergo some degree of plasticity, as pigs experience a dramatic change in environmental conditions, especially in food availability and quality. For example, domestic pigs appear to be less able to adapt their foraging strategies in varying environments (Gustafsson et al., 1999). Further, it is likely that some traits, particularly those optimized for farm conditions or production, provide little benefit or are even maladaptive under more natural conditions. Counterintuitively, it is predicted that the introduction of such maladaptive traits from domestic to wild populations could persist for long periods and pose a migration load (i.e., fitness loss to population as a result of individuals entering the population that have maladaptive genes) on wild populations (Tufto, 2017).

In general, factors limiting or facilitating establishment of wild pig populations in North America are not well understood. Introductions with five or less individuals had low (< 6%) probability of establishing new populations (Miller et al., 2021). Although the average number of pigs escaping during escape events is not known, it is likely to be temporally and geographically variable. Climate and weather play a role in driving the distribution of wild pigs. For example, winter temperature can limit or slow the establishment of wild pigs into new areas with colder climates, while summer temperatures can drive where wild pigs establish at smaller spatial scales by influencing habitat preferences (e.g., wooded areas). Wild pigs, however, have continued to spread into colder climates (Snow et al., 2017), including across large portions of Canada, suggesting that the effects of climate are less limiting on expansion than historically perceived. Predators could affect establishment success, but their effects are not well studied and are expected to be relatively minor. Although no specific traits have been implicated, it is possible that some phenotypic traits contribute to establishment success of wild pig populations.

Pigs with the IGA

The IGA does not impart any phenotypic characteristics that make the pigs containing the IGA any more likely to reproduce with mates (e.g., they are not more attractive to mates) or more capable of reproduction (e.g., more fertile). However, the IGA does impart resistance to PRRSV, which could improve reproductive outcomes (e.g., full term pregnancy, survival of piglets, healthy piglets) of pigs that are homozygous for the IGA compared to pigs without the IGA that are infected with PRRSV. However, if there is no PRRSV in the wild population (prevalence is zero), there is no benefit for possessing the IGA and no fitness advantage. As discussed in Section 6.2, the prevalence of PRRSV in the wild remains extremely low in North America, with regional studies estimating prevalence anywhere from 0-3.4% (Baroch et al., 2015; Corn et al., 2009; Pedersen et al., 2018) (Table 6-1). Therefore, under natural conditions, the IGA is expected to confer little to no fitness advantage that results in improved reproduction in wild pig populations. Thus, the likelihood of reproduction of pigs with the IGA is expected to be the same as that for pigs without the IGA.

If the likelihood of reproduction is the same for pigs with the IGA and pigs without the IGA, then the likelihood of establishment would be the same as well. To demonstrate this concept, population modeling was conducted, which is summarized in Section 6.4 and described in detail in a report contained in Appendix A, titled "Simulated fate and effects of the IGA in wild pig populations." This population model predicted the frequency of the IGA and any resulting increase in wild pig population size under a variety of conditions. In the case of an introduction to a large or growing initial wild population, the frequency of the IGA was quickly diluted by the many wild-type alleles, substantially limiting the probability that pigs within the population inherit two copies of the IGA, which is required for PRRSV resistance. When introduced into a small wild pig population, the frequency of the IGA did increase, but did not result in an increase in the wild pig population size as compared to an introduction of a pig without the IGA. In cases where there is no existing wild population and an introduction would result in the founding of a new population, there would be no PRRSV previously existing in the environment. If there is no PRRSV, there is no benefit for possessing the IGA. Therefore, in all cases, the likelihood of establishment of a population of domestic pigs possessing the IGA would be no different than a population of domestic pigs that do not possess the IGA.

Based on the information provided above, the likelihood of reproduction and establishment of pigs with the IGA is expected to be the same as that for pigs without the IGA.

6.2.4 Conclusion on exposure

Exposure is defined as establishment and/or presence of pigs with the IGA in the environment. In order for the pigs with the IGA to be present in the environment, they must escape the farm and survive in the wild. In order for the pigs with the IGA to establish in the environment, they must then disperse and reproduce with conspecifics, either other escaped pigs with or without the IGA or wild pigs. Thus, in order to determine the likelihood of exposure [(*P(E)*)] to pigs with the IGA, the likelihood of escape, survival, dispersal, reproduction and establishment was considered.

The IGA confers resistance to PRRSV in pigs, which improves their survival, reproduction and overall health compared to pigs without the IGA, but only when infected with the virus. The IGA does not impart any other fitness advantages related to the pig's ability to escape, disperse, survive (e.g., more likely to evade predators), reproduce (e.g., more attractive to mates) or establish. When the prevalence of PRRSV is zero, the pigs with the IGA have no greater fitness than pigs without the IGA that escape farms in the United States and there is no fitness advantage for possessing the IGA. If there is no PRRSV, there is no benefit for possessing the IGA. However, when the prevalence is low in wild pig populations (2-5%), there may be a small fitness advantage at the individual level; however, this advantage does not affect the overall frequency of the IGA or growth at the population level. This was corroborated with population modeling presented in Appendix A and described in Section 6.4 below. Thus, the likelihood of exposure of pigs with the IGA is no different than pigs without the IGA.

6.3 Likelihood of harms resulting from exposure

Domestic pigs without the IGA and wild pigs

The economic and ecological impacts of wild pig populations are substantial and wide-ranging. The combination of damage and control efforts costs the United States more than $1.5 billion each year.[15] The damage results primarily from the rooting, trampling, wallowing, and feeding behaviors of wild pigs (Mayer & Brisbin, 2009; USDA, 2020a). In developed areas, these behaviors result in the destruction of landscaping, golf courses, recreational fields, cemeteries, parks, lawns, and national historic sites (Mayer & Brisbin, 2009; USDA, 2020a). In agricultural areas, wild pigs destroy cropland primarily through trampling (Mayer & Brisbin, 2009) and have been shown to target sugar cane, corn, grain sorghum, wheat, oats, peanuts, and rice crops (USDA, 2020a). In addition to the destruction of crops, wild pigs have damaged farm structures including fences and irrigation systems (USDA, 2020a).

Pig behaviors also damage natural areas, including wetlands and forestlands. In wetland areas, rooting behaviors destabilize surface soils, causing erosion and sedimentation (Mayer & Brisbin, 2009). The resulting siltation as well as contamination from wallowing behaviors degrades water quality in the surrounding area, which can result in the stimulation of algal blooms and the decline of aquatic species, including fish and freshwater mussels, and insects (Mayer & Brisbin, 2009; USDA, 2020a). Wild pigs alter forestland by exposing and destroying roots, reducing species richness in the understory, and hindering the ability of the forest to regenerate by rapidly extracting seedlings (Mayer & Brisbin, 2009; USDA, 2020a). Once natural areas have been altered by wild pig behaviors, they are vulnerable to erosion, pest species, and non-native invasive plant species (Mayer & Brisbin, 2009; USDA, 2020a).

In addition to the destruction of habitats, the presence of wild pig populations can adversely impact other species through predation, competition, and disease transmission. Wild pigs prey on a number of animal species, most notably on the eggs and hatchlings of reptiles and ground nesting birds (Mayer & Brisbin, 2009; USDA, 2020a). They also compete with other species for resources including food (i.e., seeds, plants, and small animals), water, and habitat (USDA, 2020a). Wild pigs also act as vectors for disease and are known to carry at least 30 important viral and bacterial diseases and close to 40 parasites that can be transmitted to other animals and humans (Mayer & Brisbin, 2009; USDA, 2020a). In the United States, the main viruses that wild pig populations harbor are pseudorabies virus (PRV), foot and mouth disease, and African Swine Fever virus (ASFV). Common bacterial diseases in feral populations are brucellosis, tuberculosis, and leptospirosis. There are also several parasitic diseases caused by protozoa (e.g., *Giardia*) and helminths (e.g., *Trichinella* spp.) that commonly infect wild pig populations. The most common source of these infections is humans, but wild pigs can serve as secondary reservoir hosts and contribute to the spread of infection through groundwater or undercooked meat (Mayer & Brisbin, 2009).

Through the combination of these damaging effects, wild pig populations have played a role in the decline of close to 300 native plant and animal species in the United States (USDA, 2020b). Over 250 of these species are threatened or endangered, and include the sea turtle, prairie chicken, Houston toad, Mead's milkweed, Hine's emerald dragonfly, and Hawaiian moorhen (USDA, 2020b). Pigs are not native to North America, so there is no concern for impact to threatened or endangered native pig populations.

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[15] https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/operational-activities/feral-swine/feral-swine-damage; accessed August 30, 2023.

Pigs with the IGA

The second half of the risk equation is evaluating the likelihood of harm assuming exposure has occurred [$P(H|E)$]. As described above, wild pigs can cause substantial and extensive damage, both economical and physical, to various resources in the United States.

There are four ways the IGA could contribute the severity or likelihood of potential environmental harms of wild pig populations, assuming exposure occurs. The mostly likely way that the IGA could affect the potential harm occurring from pigs in the affected environment is indirectly by increasing wild pig populations, thereby increasing ecosystem damage and disease transmission. To achieve this, the IGA would need to impart increased fitness with regard to survival and reproduction. As discussed in Section 6.2, this is unlikely to occur. There is no indication that PRRSV currently has any significant effect on limiting wild pig population growth. If PRRSV were to be eliminated from North America, there is no indication that this would substantively alter the rate of establishment and population growth of wild pigs in North America (R. Miller, personal communication, 2022). Hunting, access to food resources, and climate are likely to remain the primary factors limiting the population growth rate of wild pigs in North America. Thus, any fitness advantage conferred by the IGA is expected to have a negligible influence on wild pig population growth rate. To demonstrate that wild pig population growth due to introgression of the IGA would not be expected under a variety of conditions, population modeling was conducted, which is summarized under Section 6.4 and described in detail in a report contained in Appendix A, titled “Simulated fate and effects of the IGA in wild pig populations.”

Another way the IGA could impact the harms of pigs with the IGA is directly, by altering non-target phenotypic traits that affect behaviors associated with damage from wild pigs. However, as previously discussed (see Section 3), information on the phenotypic and molecular characterization of the IGA demonstrate that pigs that contain the IGA are not expected to differ from pigs that do not contain the IGA for any traits that could exacerbate damage from wild pigs—i.e., rooting, trampling, wallowing, or metabolism (that could affect feeding rates)—and therefore present no greater likelihood for harm than domestic pigs that do not contain the IGA.

The third factor that could impact the potential harms of pigs containing the IGA is an increase of the introgression of other domestic traits to wild populations. Theoretically, if individuals with the IGA experience higher relative fitness than that expected for domestic pigs that do not contain IGA, the IGA could help to facilitate introgression of other genetic variations developed in domestic pigs, such as litter size or growth rate. The potential for this outcome depends on the fitness advantage and the genetic basis and architecture of both the IGA and the other domestic traits of concern. The fitness advantage of individuals possessing the IGA is expected to be low to non-existent, as discussed previously. However, for the rare occasions when individuals are homozygous for the IGA and there is some fitness advantage for being resistant to PRRSV (e.g., if the prevalence of PRRSV in wild pig populations dramatically increases), there could be increased potential for introgression of other traits of concern. Based on what is known about the genetic basis and architecture of such traits, this seems unlikely. These traits are multigenic (i.e., many genes are involved in these traits), polymorphic (i.e., there is variation within these genes), have relatively low heritability, and are not in close physical proximity to the IGA in the pig genome (Zak et al., 2017). For example, litter size, a trait of greatest concern regarding reproduction, is controlled not by one or even a few genes of large effect, but by many genes of small effect (Zak et al., 2017). Also, the genes involved in litter size are distributed across multiple chromosomes throughout the pig genome and none are in close physical proximity to the IGA,[16] dramatically reducing the strength of any potential indirect selection pressure provided by the IGA. Last, it is not clear to what degree environmental conditions (wild vs. farm) would alter any putative fitness advantage of these traits, as previously discussed. Such a scenario assumes that domestic traits are universally advantageous in the wild, but this is not likely to be the case. Trade-offs[17] are already known to exist under farming conditions. For instance, selection for increased ovulation rate and number of fetuses can result in an increase in numbers of stillborn and mummified pigs and selection for increased birth weight can cause a reduction in litter size (Johnson et al., 1999). These trade-offs likely also exist in the wild and novel trade-offs emerge, when these traits are exposed to wild conditions where resource availability, environmental conditions, and other factors differ considerably from farm conditions.

Last, there is the possibility that the presence of the IGA on farms, even without the escape of pigs with the IGA into the wild, could lead to environmental harms. Given that the spread of PRRSV is thought to be primarily unidirectional—from domestic to wild pigs (Pedersen et al., 2018)—a reduction in PRRSV resulting from the use of pigs that contain the IGA on farms could have the effect of reducing transmission of PRRSV to wild populations, and in turn reduce the prevalence of PRRSV in the wild. This could result in an increase in wild pig populations and an increase in ecosystem damage or disease transmission. However, as discussed above, PRRSV has not been demonstrated to be a major factor limiting wild pig populations (R. Miller, personal communication, 2022; VerCauteren et al., 2019). Therefore, a reduction in PRRSV in wild pig populations would not be expected to impact the potential harms to the environment resulting from exposure to pigs containing the IGA.

Thus, the environmental harms, and the likelihood and severity of those harms, are not expected to be any different between pigs with the IGA and domestic pigs without the IGA.

6.4 Simulation modeling

Based on the information presented in this EA, there is nothing about the IGA that would be expected to change the likelihood of exposure or the potential harms to the affected environment due to exposure of the pigs with the IGA or the severity and likelihood of those harms occurring. These determinations were corroborated through simulation modeling, which indicates that under a broad range of environmental conditions and escape scenarios (see Appendix A, Table A-6), an escape of pigs that contain the IGA had the same effect on wild pig population growth rate as domestic pigs that do not contain the IGA (see Appendix A, Figures A-9 through A-16). This was true even for scenarios developed to reflect conservative conditions and reasonable worst-case scenarios, such as a small initial wild population (600 pigs), a high prevalence of PRRSV (5%), and an escape of 600 pigs into the wild every 5 years (simulating a large natural disaster).

Simulations also confirmed that the frequency of the IGA and the homozygous genotype would behave similar to neutral genetic variation (i.e., genes that have no fitness effects). Under most conditions, this results in the homozygous genotype (IGA/IGA) remaining at extremely low frequency or decreasing over time, or both, following an escape event. Only under the reasonable worst-case scenarios simulating disasters (escape of 600 pigs) in small initial wild

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[16] https://www.animalgenome.org/cgi-bin/QTLdb/SS/traitmap?trait_ID=157&traitnm=Total%20number%20born%20alive; accessed August 31, 2023.

[17] A trade-off exists when a change in one life history trait that increases fitness is coupled to a change in another life history trait that decreases fitness, resulting in a negation of any potential fitness advantage. For example, higher fat deposition could be advantageous to pigs living in cold climates by providing extra insulation from the cold but be disadvantageous in warm climates where it could result in overheating.

pig populations (600 pigs), or when there were repeated large escapes into a small stable population, did the frequency of the IGA/IGA genotype increase to a greater extent than neutral genetic variation. Even though the IGA/IGA genotype increases in frequency in these scenarios, there was no corresponding increase in wild pig population size as compared to the introduction of domestic pigs without the IGA. These reasonable conservative and reasonable worst-case scenarios are also unlikely to occur repeatedly in the same population as was the condition (assumption) in the model and instead would likely randomly occur among wild populations. Further, the model assumed PRRSV prevalence to be fixed and thus the fitness advantage for possessing the IGA/IGA genotype to remain constant. However, under conditions where the homozygous genotype would be favored (i.e., high prevalence of PRRSV), the increase in the IGA allele in the wild population would likely result in negative feedback to reduce the prevalence of PRRSV and, thus, any fitness advantage conferred by the IGA.

Thus, based on reasonably conservative model assumptions and scenarios, simulation modeling indicates the IGA allele is not expected to alter wild pig population growth rates if pigs containing the IGA were to escape into the wild, even in worst-case scenarios, such as a large release during a natural disaster. Thus, there is not expected to be any more environmental harm from escaped pigs with the IGA than pigs without the IGA.

6.5 Potential toxicity due to the IGA

In addition to evaluating the environmental risk from pigs with the IGA, the potential environmental risk of the IGA itself was considered. Potential harm from the IGA could include toxicity to the environment from presence of the IGA in waste, or secondary toxicity to non-target predators and scavengers in the environment that consume tissues from pigs with the IGA.

As described in Section 3.3 above, the IGA consists of a double strand break within the targeted region of the *CD163* gene that is stably integrated into the genome of domesticated pigs and no hazards with the IGA itself have been identified (e.g., new expression products, presence of antimicrobial resistance markers). Therefore, disposal of the waste material (e.g., land application, composting, and incineration in manure and carcasses) associated with the production, processing, and consumption of the pigs that contain the IGA would not require handling that is different from that used for domestic pigs that do not contain the IGA. Disposal practices are expected to follow local regulations, as described under Section 4.1.4.

In addition, FDA completed a food safety evaluation to consider whether food derived from pigs with IGA is as safe as food from pigs without the IGA, including an evaluation of whether there are risks of consumption of pigs with the IGA by other animals as part of animal feed. FDA did not identify any concerns from consumption of the IGA in the pigs by domestic animals; thus, there are no concerns regarding secondary toxicity to non-target animals in the environment that may consume escaped pigs with the IGA. Details on the food safety evaluation can be found in the FOI Summary that will be made available on AnimalDrugs@FDA.

6.6 Risk of harm to the environment

Ultimately, no hazards have been identified from the IGA itself and the IGA is not expected to impart any fitness advantage to domestic pigs that could impact the likelihood of exposure or the severity or likelihood of harm to the environment. Following escape of pigs containing the IGA into wild populations, three separate conditions must be met simultaneously to confer a fitness advantage, each with its own very low likelihood of occurring (Figure 6.3). The first condition is that individuals must be homozygous for the IGA to have a fitness advantage. The second condition requires that, for any fitness advantage to be realized, the prevalence of PRRSV in the wild would have to be substantially higher than the current prevalence (0-3.4%; Table 6-1). Last, a substantial portion of the individuals in the wild would need to be homozygous for the IGA, which as discussed in Section 6.1, is very unlikely to occur.

Because the IGA imparts little to no fitness advantage to the animal, the likelihood of exposure of pigs with the IGA was determined to be unknown and variable, but the same as that for domestic pigs without the IGA. In addition, the potential harms to the United States and the severity and likelihood of those harms occurring, assuming exposure, was also determined to be no different from domestic pigs without the IGA. Therefore, the risk of potential harm to the United States environment from the pigs with the IGA is no different from that of domestic pigs without the IGA and no significant impacts to the human environment are expected from the approval of the original application for the IGA contained in pigs intended to confer resistance to PRRSV for use throughout the United States.

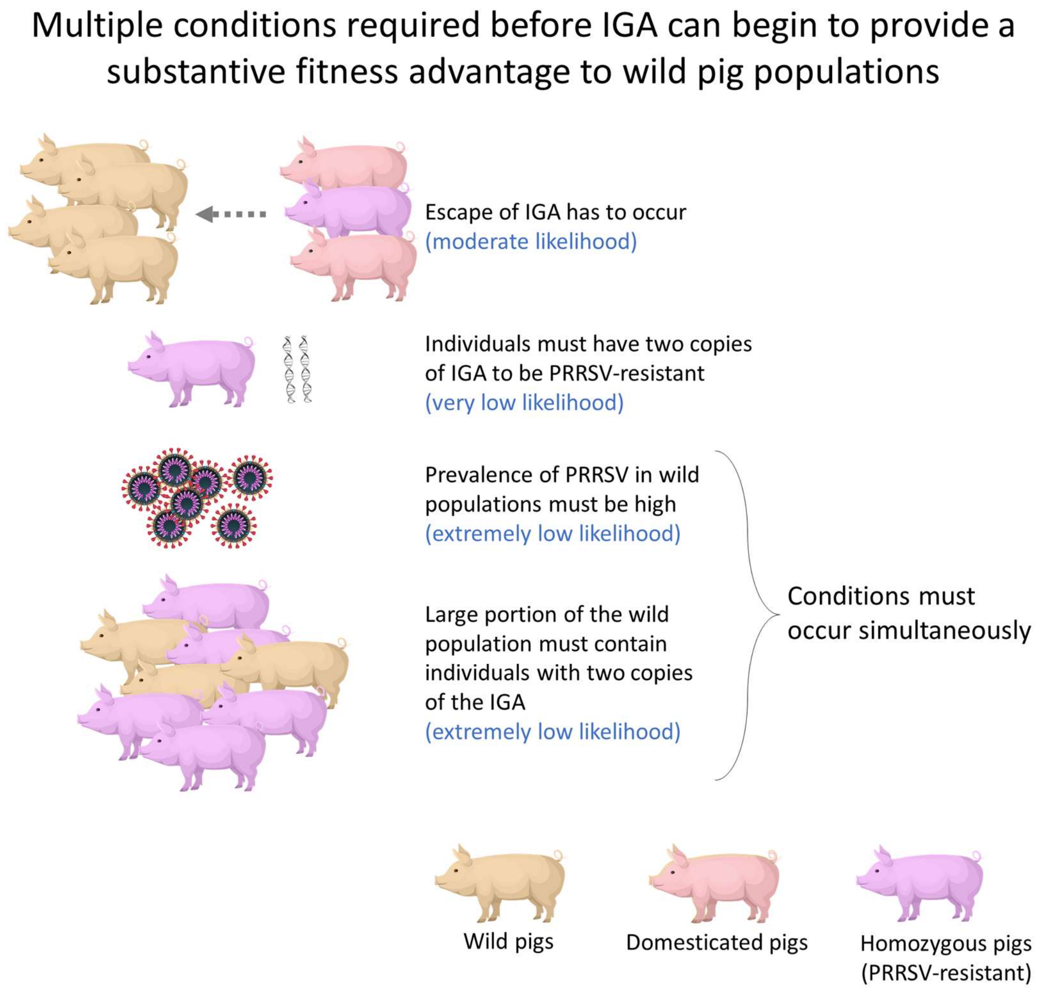

Figure 6-3. **Overview of the multiple conditions required before the IGA can begin to provide a substantive fitness advantage to wild pig populations.**

7. ALTERNATIVE TO THE PROPOSED ACTION

The only alternative to the proposed action is the “No Action” alternative, which would be the failure to approve the original application for the IGA, the deletion of exon 7 of *CD163* gene. Under the “No Action” alternative, Genus would discontinue the development and production of PRRSV-resistant pigs and no significant impacts on the quality of the human environment in the United States would be expected. However, based on the analysis in this EA, significant environmental impacts are not expected to occur from the proposed action. Therefore, the “No Action” alternative was eliminated from consideration and further analysis.

8. AGENCIES AND PERSONS CONSULTED

This EA was prepared with input from the Center for Veterinary Medicine at FDA and from Dr. Ryan Miller of the USDA Center for Epidemiology and Animal Health.

9. LIST OF PREPARERS

This document was prepared by Exponent, Inc., under the direction of Sean Ryan.

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Appendix A

Simulated fate and effects of the IGA in wild pig populations

Model Overview

Model background

The potential fate and effects of the IGA during an escape event was modeled using the most recent version of SLiM, version 4.0.1 (Haller & Messer, 2023). SLiM is a genetically explicit individual-based, forward-time population genetic simulation framework. It has been used extensively to answer a broad range of questions and has been cited in more than 800 publications since 2017, including numerous studies evaluating the risk of gene drive technologies (e.g., J. Champer et al., 2020, 2021; S. E. Champer et al., 2020; Combs et al., 2023). That is, SLiM allows one to simulate a population of animals (e.g., pigs) through time in an environment that can be varied (e.g., variation in PRRSV prevalence), where every individual is tracked, can evolve, and has a set of life history traits (e.g., litter size, maximum life span, gestation period, castration status, likelihood of survivorship by age) that can vary by individual. The National Institutes of Health's Compare Simulators by Attribute tool[18] lists SLiM as the most comprehensive among the simulation software reviewed. Thus, SLiM is an ideal modeling framework to evaluate the potential fate and effects of the IGA and its potential effect on wild pig population growth rate.

Model development

A non-Wright Fisher model was used to simulate wild and managed populations containing male and female diploid individuals because this type of population genetic model allows for overlapping generations (parents can live at the same time as their offspring) and population size is not fixed but allowed to vary. The model was run in time-steps of 1-month intervals. Each month any individual could become infected, reproduce, escape, or die, or experience a number of these events (Figure A-1). The model was non-spatial with two populations simulated—a wild population and a farm population (i.e., individuals that may have the IGA). The farm population was created solely to provide a source for introductions into the wild, whether escaping from a farm or during transportation. Escapes were allowed to vary in frequency and size. At each time-step (month), the frequency of the IGA/IGA (homozygous) genotype in the wild population and the total size of the wild population was recorded.

Prior to any event or time-step in the model, all constants and parameter values are initialized. That is, all parameter values that will be used in the simulation are input into the model.

The initial event in the simulation that occurs prior to the first time-step creates the wild and farm populations, introduces the IGA to the farm population, and introduces PRRSV to the wild population (the percent of individuals that are infected is equal to the expected prevalence percentage) (Figure A-1, "Setup conditions").

For each time-step in the model, each individual has the opportunity to reproduce, then their status is updated based on survivorship which depends on natural mortality, carrying capacity, and probability of infection (Figure A-1, "Reproduction", "Survivorship, infection, and carrying capacity"). In the reproduction event, each female may reproduce, based on whether a male is available, whether she is pregnant, and the probability of conceiving. If the reproduction event is successful, she produces offspring, each a unique individual in the model that will then be tracked and evaluated in subsequent time-steps. Probability of conception and number of litter (offspring) produced is adjusted based on her infection status—a penalty is applied to PRRSV-

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[18] https://surveillance.cancer.gov/genetic-simulation-resources/search/; accessed August 21, 2023.

infected females, so they are potentially less likely to conceive, and if they do conceive, likely to produce fewer offspring.

In the survival, infection, and carrying capacity events, the probability of survival is determined by the age (i.e., natural mortality) and infection status of the individual. For scenarios where carrying capacity (i.e., the maximum number of organisms that can be supported in a given area or habitat)[19] is implemented, survivorship is adjusted based on current population size as represented by the following equation:

$$individual\ survivorship = current\ probailty\ of\ survivorship\ x\ (\frac{carrying\ capacity}{current\ population\ size})$$

Simulations were conducted either with or without carrying capacity based on whether the population size of wild pigs are limited. Both assumptions are realistic since, like most populations, wild pigs could be limited due spatial constraints and resource availability. However, since wild pigs are invasive, they often outcompete native species and can grow and spread nearly without bound.

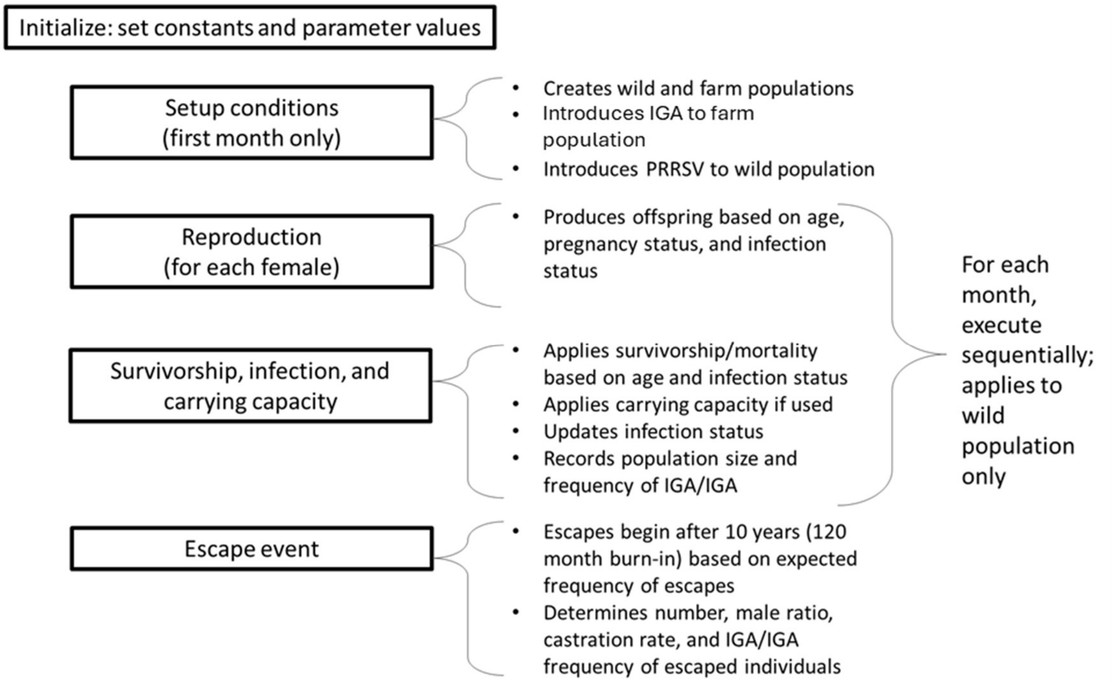

Figure A-1. Overview of events in the simulation model.

In terms of disease dynamics, susceptible individuals (i.e., those not infected nor homozygous for the IGA) are then infected proportional to the expected prevalence of PRRSV. In the last event of each time-step (Figure A-1, “Escape event”), an escape occurs based on the expected frequency of escape. The conditions of escape—number of individuals, male ratio, percent castrated, frequency of IGA/IGA genotype—is determined and these individuals are “released” into the wild population.

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[19] Lincoln, R., Boxshall, G. and Clark, P., 1998. A dictionary of ecology, evolution and systematics.

There were 53 parameters in the model (Table A-1) in total. The values for these parameters were either point estimates that did not vary (e.g., fixed values such as mutation rate, type of mutation) or were randomly drawn from normal or uniform distributions. The values used for these parameters were determined from an extensive review of the literature (see references in Table A-1).

Table A-1. Parameters used in the simulation model. Note that many parameters listed are composed of two separate parameters (i.e., min and max or mean and standard deviation (sd)) but organized into one for simplicity.

Parameter	Units	Distribution	Description	Reference
Escape interval	years	point	frequency an escape event occurs	Based on discussions with CVM
Escape size	individuals	point	number of individuals escaping	
Frequency of IGA/IGA on farms (min and max)	percent	uniform	frequency of IGA/IGA genotype on a farm	Estimated by Genus; based on expected use of the product
Percent of escaped males (min and max)	percent	uniform	percent of males in an escape event	Unknown
Percent of escaped males castrated (min and max)	percent	uniform	percent of castrated males in an escape event	Rault et al., 2011
Maximum age of wild pigs (min and max)	years	uniform	maximum age of a wild pig	Barrett, 1978; Henry & Conley, 1978; VerCauteren et al., 2019
Prevalence of PRRSV (start)	percent	point	prevalence of PRRSV in the wild at the start of the simulation	Baroch et al., 2015; Corn et al., 2009; Pedersen et al., 2018; Saliki et al., 1998; Wyckoff et al., 2009
Prevalence of PRRSV (end)	percent	point	prevalence of PRRSV in the wild at end of the simulation	
Duration of PRRS (min and max)	months	uniform	duration of infection of PRRS (fitness effects) in the wild	Dee, 2022; Dewey, 2000; Global Ag Media, 2018; Iowa State University, 2023; Wills et al., 2003; World Organisation for Animal Health, 2008; Zimmerman et al., 2019b
Initial wild population size	individuals	point	initial size of the wild population	see Table A-5
Carrying capacity of wild population	individuals	point	carrying capacity for the wild population; equal to the initial population size	NA
Whether to use carrying capacity	-	binomial	determines whether to use carrying capacity in the model	NA
Whether natural resistance exists	-	binomial	determines whether recovered individuals become resistant or susceptible	NA

Parameter	Units	Distribution	Description	Reference
Age at sexual maturity (min and max)	months	uniform	age at sexual maturity	Belden & Frankenberger, 1990; Mayer & Brisbin, 2009; Reiland, 1978; Sweeney et al., 1979; VerCauteren et al., 2019
Gestation period (min and max)	months	uniform	gestation period of a wild female pig; duration of the prenatal period	VerCauteren et al., 2019
Litter size of juveniles (mean and sd)	individuals	normal	number of offspring produced by a juvenile	Chinn et al., 2022; Taylor et al., 1998; VerCauteren et al., 2019
Litter size of yearlings (mean and sd)	individuals	normal	number of offspring produced by a yearling	
Litter size of adults (mean and sd)	individuals	normal	number of offspring produced by a adult pig	
Conception rate (min and max)	percent	uniform	success rate of breeding in wild pigs	Chinn et al., 2022; VerCauteren et al., 2019
PRRS conception penalty (min and max)	percent	uniform	decrease in the likelihood of conception for infected females	Wensvoort, 1993
PRRS litter size penalty (min and max)	percent	uniform	percent of offspring (litter) lost due to PRRS for infected individuals	Hopper et al., 1992; Keffaber, 1989; Loula, 1991; Zimmerman et al., 2019a, 2019b
PRRS survivorship penalty for juveniles (min and max)	percent	uniform	PRRS-induced mortality for juveniles	Moore, 1990; Zimmerman et al., 2019a, 2019b
PRRS survivorship penalty for yearlings (min and max)	percent	uniform	PRRS-induced mortality for yearlings	
PRRS survivorship penalty for adults (min and max)	percent	uniform	minimum PRRS-induced mortality for adults	
Background/Natural mortality (mean and sd)	percent	normal	background mortality for all age classes	VerCauteren et al., 2019
Age of farm animals (min and max)	months	uniform	age range of pigs in farm population	Age of farm animals can vary widely among farms. Therefore, animal age reflected the same age range (min and max) used for wild animals.
Prenatal development period	months	point	end of prenatal stage	This parameter is determined based on the “gestation period” parameter set in the model.
Juvenile development period	months	point	start and end of juvenile stage	VerCauteren et al., 2019
Yearling development period	months	point	start and end of yearling stage	VerCauteren et al., 2019
Adult development period	months	point	start of adult stage	VerCauteren et al., 2019

Parameter	Units	Distribution	Description	Reference
Initial size of farm population	-	point	used solely to create the farm population	NA
Mutation rate	-	point	set mutation rate	Set to zero so as not to produce the IGA naturally
Recombination rate	-	point	set recombination rate	Default in SLiM; similar to 0.95 cM/Mb observed in Johnsson et al., 2021
Genomic element	-	-	region of the chromosome with the mutation	NA
Sex	-	-	allows males and females and sexual reproduction (mating)	NA
Mutation type	-	-	type of mutation (e.g., homozygous recessive)	NA

Sensitivity Analysis

Purpose

A sensitivity analysis was performed to assess the robustness of the simulation model and to identify parameters within the model that have the greatest effect on model outcomes.

Background

Sensitivity analysis is a fundamental component of modeling, widely recognized for its importance in assessing the reliability and robustness of model outputs (Saltelli et al., 2008). It plays a critical role in various domains, including environmental modeling (Pianosi et al., 2016) and engineering (Douglas-Smith et al., 2020). Sensitivity analysis aids in identifying influential factors, quantifying uncertainties, validating and improving models, and supporting risk assessment and management (Pianosi et al., 2016; Saltelli et al., 2008). By considering variations in input parameters and assessing their impact on model outcomes, sensitivity analysis provides valuable insights into the behavior and performance of models under different conditions (Douglas-Smith et al., 2020). Overall, sensitivity analysis is essential for improving the interpretation and reliability of models, and it serves as a valuable tool for the decision-making processes (Douglas-Smith et al., 2020; Pianosi et al., 2016; Saltelli et al., 2008).

Experimental design

The primary purpose of the sensitivity analysis was to evaluate the relative sensitivity of model outputs from the SLiM model—specifically the wild pig population size and the proportion of genotypes homozygous for the IGA/IGA (PRRSV resistant phenotype) within the wild population—to changes in model inputs (parameters).

Of the 53 parameters in the model, 18 were varied for the sensitivity analysis (Table A-2); the other parameters remained fixed (i.e., used a single value or distribution). The SLiM model was run using a full factorial combination of the 18 parameters, each using the min and max value observed in published studies. This resulted in a total of 262,144 unique parameter combinations. Each set of parameter combinations was run using 10 unique seeds[20] to generate

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[20] Starting value for the random number generator.

10 replicates for each set of parameter combinations (a total of 2,621,440 simulations). For natural history parameters, these values were restricted to studies from the United States, so the parameter estimates reflect wild pig populations in the United States. For PRRSV-related parameters, estimates were taken from studies of farm populations (not necessarily specific to the United States), with the exception of the prevalence of PRRSV in wild pig populations, which was based on multiple studies from the United States, spanning several decades. Although the average prevalence of PRRSV observed in wild pig populations in the United States is ~2%, an extremely conservative maximum value of 30% was used. A conservatively high prevalence was used in the sensitivity analysis to determine how this parameter would affect model behavior. The maximum escape size ranged from 0 to 600 (600 was used to reflect a rare natural disaster event).

Table A-2. Parameter values used in the sensitivity analysis; parameters shaded in green were varied for the analysis

Parameter	Units	Distribution	Values for Sensitivity Analysis
Escape interval	years	-	min = 1; max = 50
Escape size	individuals	-	min = 0; max = 600
Frequency of IGA/IGA on farms	percent	-	min = 0.5; max = 1
Percent of escaped males	percent	-	min = 0; max = 1
Percent of escaped males castrated	percent	-	min = 0; max = 1
Maximum age of wild pigs	years	uniform	min = 8; max = 14
Prevalence of PRRSV (start and end)	percent	-	min = 0; max = 0.3
Duration of PRRS	months	-	min = 1; max = 9
Initial wild population size	individuals	-	min = 600; max = 50000
Carrying capacity used in wild population	-	-	T; F
Natural resistance exists	-	-	T; F
Age at sexual maturity	months	uniform	min = 3; max = 12
Gestation period	months	uniform	min = 6; max = 12
Litter size of juveniles	individuals	-	min = 3; max = 5
Litter size of yearlings	individuals	-	min = 4; max = 6
Litter size of adults	individuals	-	min = 5; max = 7
Conception rate	percent	uniform	min = 0.12; max = 1
PRRS conception penalty	percent	-	min = 0; max = 0.5
PRRS litter size penalty	percent	-	min = 0.1; max = 1
PRRS survivorship penalty for juveniles	percent	-	min = 0; max = 0.2
PRRS survivorship penalty for yearlings	percent	-	min = 0; max = 0.1
PRRS survivorship penalty for adults	percent	-	min = 0; max = 0.1
Background/Natural mortality	percent	normal	mean = 0.56; sd = 0.26
Age of farm animals	months	-	min =8; max = 14
Sex	-	-	A
MUTATIONTYPE	-	-	0
PRENATAL_END	months	-	0 + GESTATION_PERIOD
JUVELINE_START	months	-	0 + GESTATION_PERIOD
JUVELINE_END	months	-	12 + GESTATION_PERIOD
YEARLING_START	months	-	12 + GESTATION_PERIOD

Parameter	Units	Distribution	Values for Sensitivity Analysis
YEARLING_END	months	-	24 + GESTATION_PERIOD
ADULT_START	months	-	24 + GESTATION_PERIOD
m2_DFE	-	-	0
ESCAPE_EVENTS	-	-	Determined in Model
K_p2	-	-	10000
Mutation rate	-	-	0
Recombination rate	-	-	-1.00E-08
Genomic element	-	-	(g1, 0, 99999)
Sex	-	-	A (allow sexual reproduction)
Mutation type	-	-	homozygous recessive

For parameter combinations where carrying capacity was used, the simulations were run for a total of 60 years (10-year burn-in),[21] while for scenarios where there was no carrying capacity, the simulation was run for a total of 20 years (10-year burn-in) due to the larger computational demand (i.e., there were a substantially greater number of individuals to track).

Simulations were split into groups for analysis based on two parameters: 1) whether carrying capacity was used in the model (yes/no), and 2) the initial wild population size used (600 or 50,000; see below, Table A-5). This resulted in four groups that were evaluated separately: noK-600 (no carrying capacity and initial wild population size of 600), noK-50k (no carrying capacity and initial wild population size of 50,000), withK-600 (with carrying capacity and initial wild population size of 600), withK-50k (with carrying capacity and initial wild population size of 50,000). This grouping was performed because in the simulation model, carrying capacity and initial wild population size are expected to substantially affect final wild population size and would dominate the relative importance of any sensitivity analysis. Therefore, the sensitivity analysis was designed to focus on evaluating the relative sensitivity of the other parameters in the model for which the effects on model outputs are less understood.

Statistical methods

The machine learning algorithm random forest was used to conduct the sensitivity analysis. The final wild pig population size and IGA/IGA genotype frequency were used as response variables, while the 16 simulation model parameters (all parameters except for carrying capacity and initial population size) were considered as predictor (explanatory) variables.

Random forest is a widely employed machine learning technique for conducting sensitivity analysis. It is an ensemble learning method that combines multiple decision trees to create a robust predictive model. Random forest has gained popularity in sensitivity analysis due to several key advantages. First, it can effectively handle high-dimensional data with a large number of input variables, making it suitable for complex systems (Cutler et al., 2007). Second, random forest provides a measure of variable importance by evaluating the impact of each input variable on the model's predictive performance, allowing for the identification of influential factors (Breiman, 2001). Furthermore, random forest models are less prone to overfitting compared to individual decision trees, providing more reliable and stable sensitivity analysis results (Cutler et al., 2007).

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[21] Initial cycles (years of data) in the simulation that allow the population to reach a stable state or equilibrium before data is collected or analyzed; these cycles are discarded prior to analysis.

The R package “randomForest” was used to conduct the sensitivity analysis. The randomForest package implements the random forest algorithm proposed by Breiman (2001) and offers flexibility in settings to tailor the analysis according to specific needs. Our random forest model aimed to predict the final size of the wild pig population and the frequency of the homozygous IGA genotype (IGA/IGA) from 16 predictors (parameters from the SLiM model). The randomForest model was configured with 100 trees, a maximum of 10 tree nodes, and 8 predictors for splitting. The predictive power of each predictor was assessed using partial least square regressions with adjusted R-squared[22] (R^2) values; observed values were SLiM model simulation outputs and predicted values were generated from the random forest simulations. The importance of each predictor was measured by determining the increase in mean squared errors (MSE) caused by random permutations of predictor values.

Results of the sensitivity analysis

Overall, the influence of the simulation model inputs on model outputs depended greatly on whether carrying capacity was used in the model. When no carrying capacity was used in the model (noK-600, noK-50k), variation in model inputs were strongly correlated with model outputs, indicated by the high R^2 values (Figure A-2). Contrastingly, in simulations where carrying capacity was used (withK-600, withK-50k), only IGA/IGA genotype frequency showed a high R^2 value while the wild pig population size had a low R^2 value, particularly for the small population where the R^2 value was near zero (Figure A-3). The low R^2 value in simulations using carrying capacity indicates that changes in model parameters have little to no effect on wild pig population size. Therefore, further evaluation of the relative sensitivity of parameters focused primarily on parameter combinations that included *no carrying capacity* (i.e., noK-600 and noK-50k). It is worth noting that the general patterns observed for the relative sensitivity of parameters for no carrying capacity were similar to those with carrying capacity (Figures A-4, A-5, A-6, and A-7).

Why the model was insensitive to parameters when carrying capacity was used is not immediately clear but could have to do with the timing (within the steps of the model) of when wild pig population size was measured/recorded. The SLiM model records wild pig population size after carrying capacity is implemented in the model. Recording population size prior to the implementation of carrying capacity could help detect model sensitivity to changes in parameters, but whether this is the case and to what extent sensitivity would increase is not known.

For the *no carrying capacity* scenarios, the sensitivity of each parameter, measured as the percent increase in the MSE (%IncMSE) was ranked from highest to lowest; the higher the %IncMSE, the greater the model sensitivity to this parameter—i.e., the greater the influence that parameter has on model outputs. A general pattern that emerged for both outcomes (population size and IGA/IGA frequency) was that parameters associated with natural history traits or conditions of escape typically ranked among the highest for %IncMSE (Figures A-4 and A-5). This was particularly true for simulations using a small wild population (600 pigs) (Figure A-5). For the outcome of wild pig population size, a notable difference between the (independent variables) small and large initial population size was that when the initial population was small, the most sensitive parameters were those associated with natural conditions of the escape (i.e., escape interval, escape size, escape male ratio, and percent of males castrated). This is not surprising given that the parameter values include the possibility of an escape of 600 pigs every

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[22] A statistical measure that indicates how much of the variation of a dependent variable is explained by an independent variable in a regression.

year, which is the initial size of the wild population. Contrastingly, for simulations where the initial population was large, the most influential parameters were related to natural history traits (i.e., reproduction), but two PRRS-related traits also exhibited modest influence—prevalence of PRRSV and duration of PRRS symptoms.

The finding that final wild pig population size was sensitive to the prevalence of PRRSV is not surprising given that an extremely conservative maximum value was used (30%). The reason why prevalence of PRRSV had a greater influence in large populations is not clear but may have to do with natural resistance to PRRSV (when set to "True" in the model) being more effective in small populations when the prevalence of PRRSV is high. Similarly, the maximum value for the parameter PRRS duration that determines how long individuals are symptomatic (and fitness consequences applied) was also very conservative—8 months (Wills et al., 2003); the average (and median) based on multiple studies in the literature is 2-4 months.[23] The use of this conservative value likely increased model sensitivity for this parameter beyond what is likely to occur under natural conditions. No significant impact of PRRSV has been observed in wild pig populations (Ruiz-Fons et al., 2008).

Regardless of initial population size (600 or 50,000), the frequency of the homozygous state of the IGA was primarily sensitive to farm conditions, particularly those related to escape conditions (Figures A-4 and A-5). Perhaps not surprisingly, of the parameters related to PRRSV, the frequency of the IGA on farm populations had the greatest effect, although the importance of this parameter was very low in large wild pig populations and relatively modest in small wild pig populations when compared to the importance of non-PRRSV related parameters.

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[23] https://swine.extension.org/porcine-reproductive-and-respiratory-syndrome-prrs/

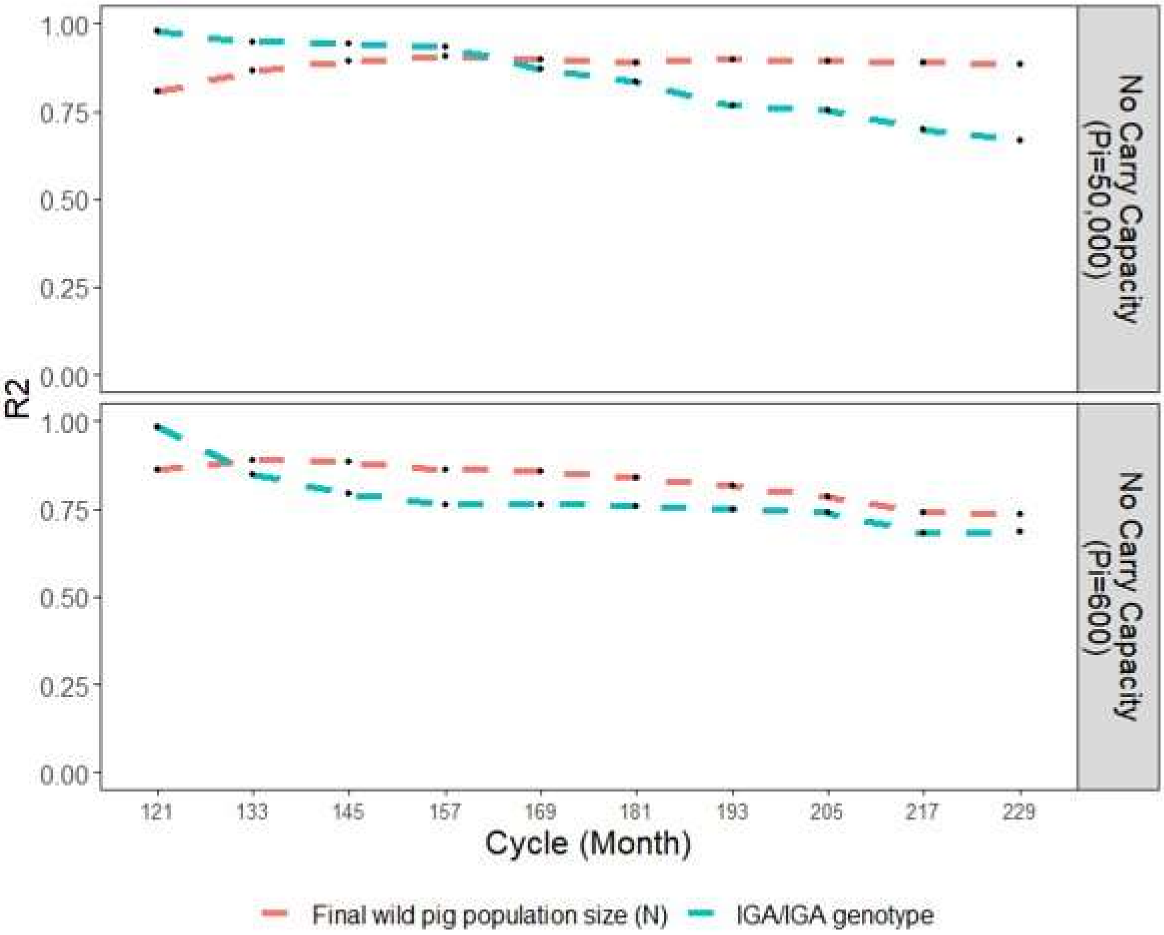

Figure A-2. **R^2 estimates of predicted (RandomForest) and observed (SLiM) estimates for all parameters for each cycle (month) in the simulation for scenarios with no carrying capacity and starting populations of 50,000 and 600. Predicted variables evaluated were wild pig population size (N) and the frequency of the homozygous state of the IGA (IGA/IGA). P_i = initial wild population size.**

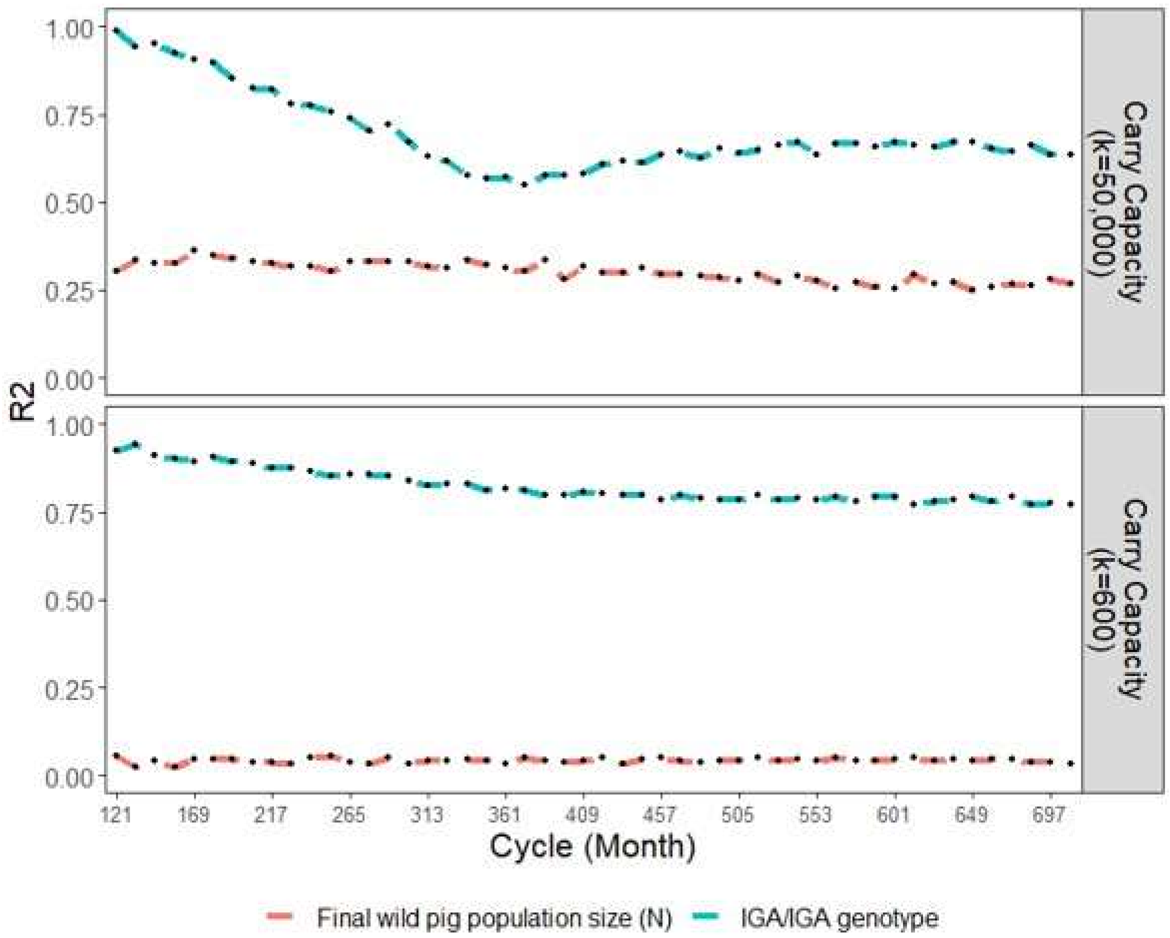

Figure A-3. R^2 estimates of predicted (RandomForest) and observed (SLiM) estimates for all parameters for each cycle (month) in the simulation for scenarios with carrying capacity and starting populations of 50,000 and 600. Predicted variables evaluated were wild pig population size (N) and the frequency of the homozygous state of the IGA (IGA/IGA). K = carrying capacity.

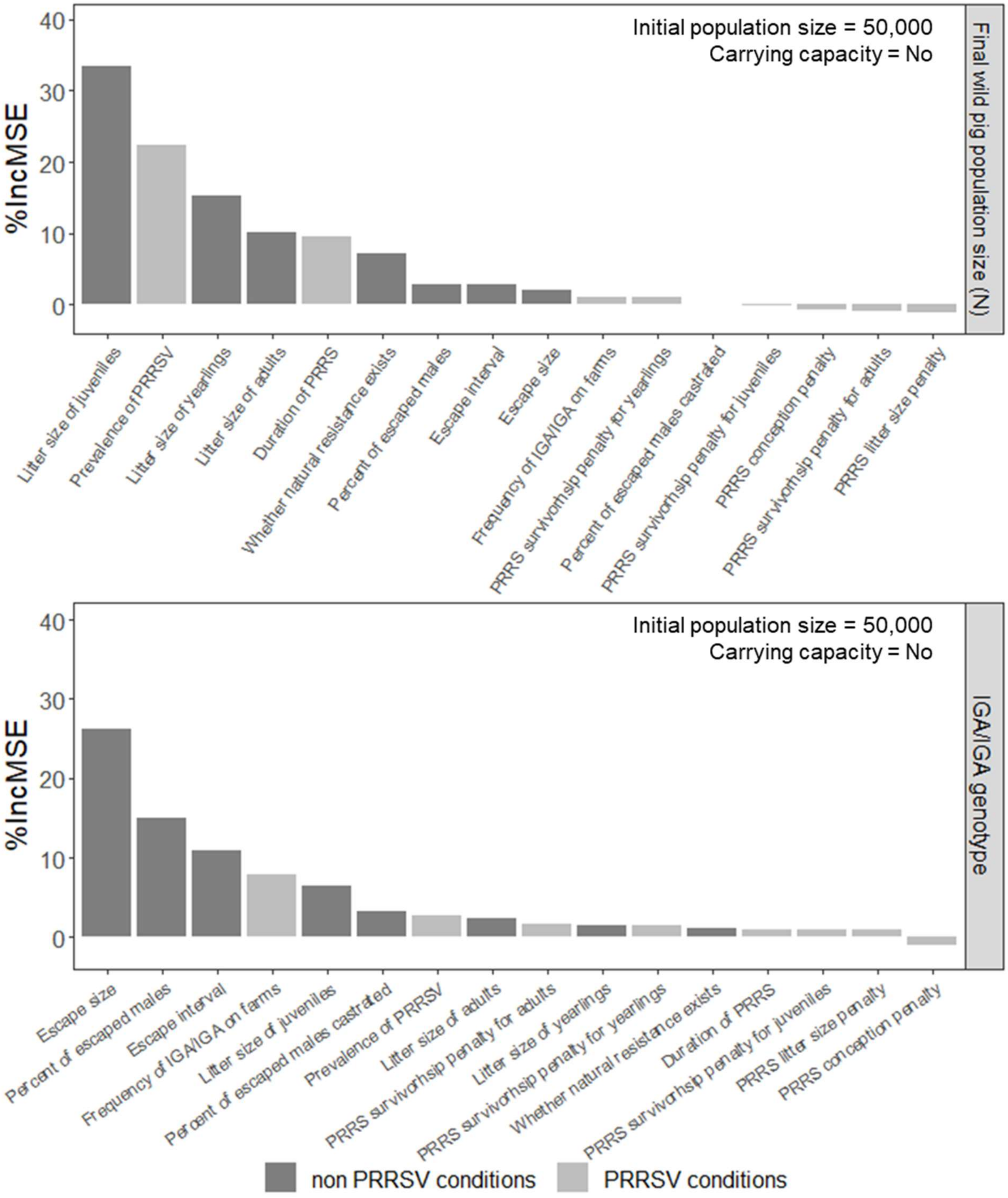

Figure A-4. **Percent increase in MSE for each parameter (predictor variable) and predicted variables (N and IGA/IGA gentoype) for scenarios with an initial population size of 50,000 and no carrying capacity. Higher values indicate greater sensitivity of predicted variables to changes in those parameters. Parameters are shaded based on whether they are parameters associated with PRRSV (PRRSV conditions) or natural, non-PRRSV related conditions (non PRRSV conditions).**

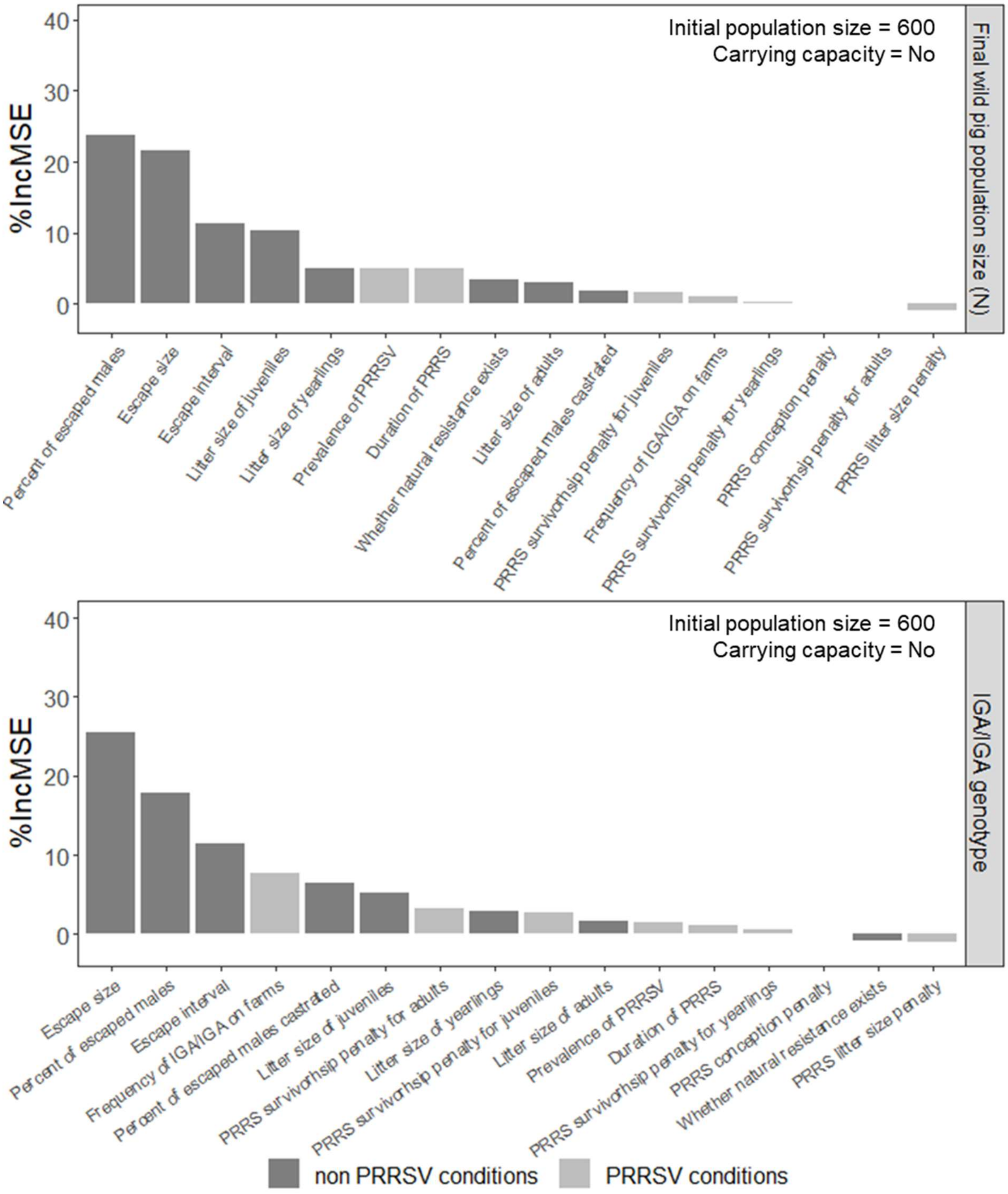

Figure A-5. **Percent increase in MSE for each parameter (predictor variable) and predicted variables (N and IGA/IGA genotype) for scenarios with an initial population size of 600 and no carrying capacity. Higher values indicate greater sensitivity of predicted variables to changes in those parameters. Parameters are shaded based on whether they are parameters associated with PRRSV (PRRSV conditions) or natural, non-PRRSV related conditions (non PRRSV conditions).**

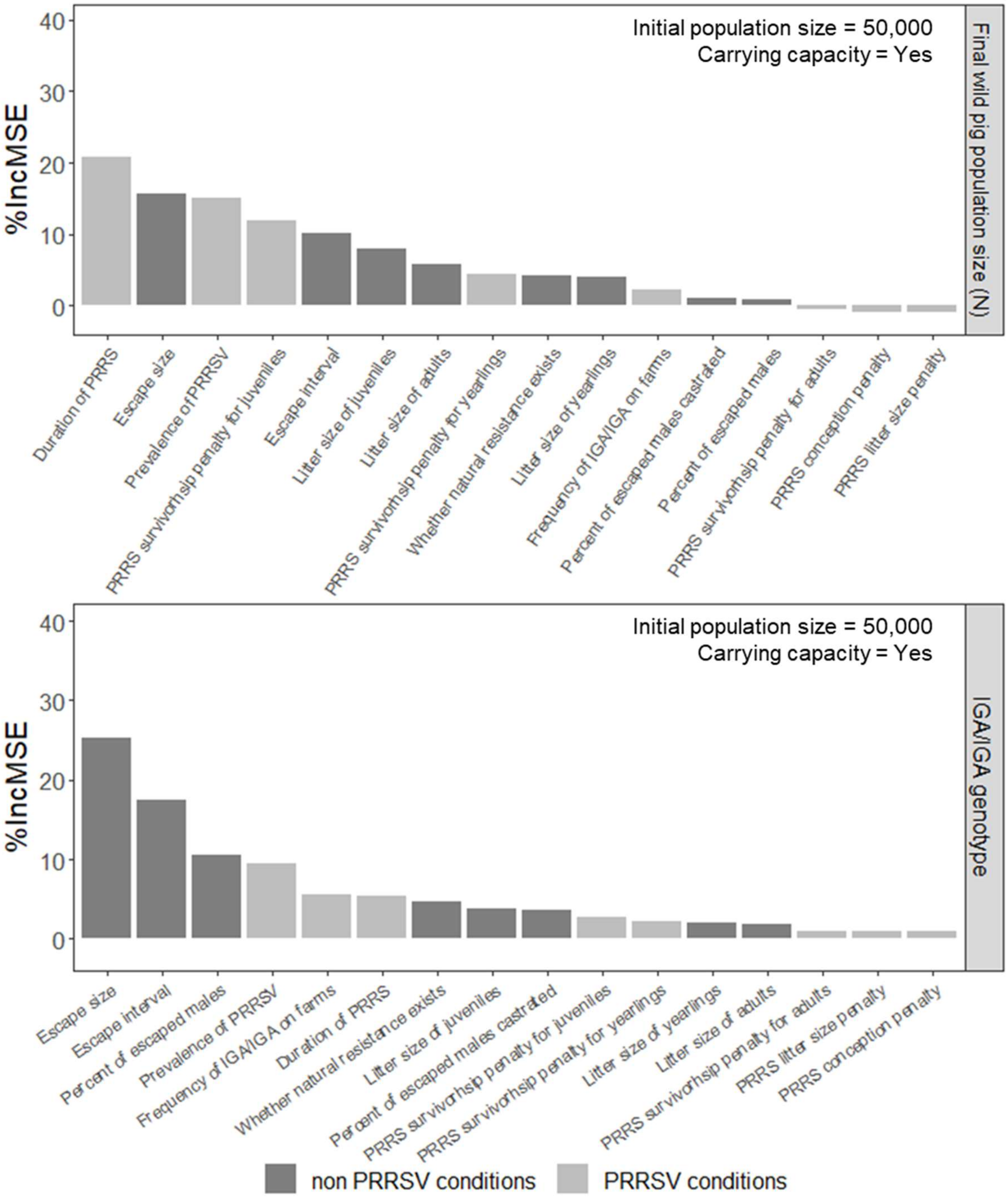

Figure A-6. **Percent increase in MSE for each parameter (predictor variable) and predicted variables (N and IGA/IGA genotype) for scenarios with an initial population size of 50,000 and using carrying capacity. Higher values indicate greater sensitivity of predicted variables to changes in those parameters. Parameters are shaded based on whether they are parameters associated with PRRSV (PRRSV conditions) or natural, non-PRRSV related conditions (non PRRSV conditions). Note, given the low R^2 for N (Figure A-3), caution should be taken when interpreting relative importance as these parameters had low power in predicting this model outcome.**

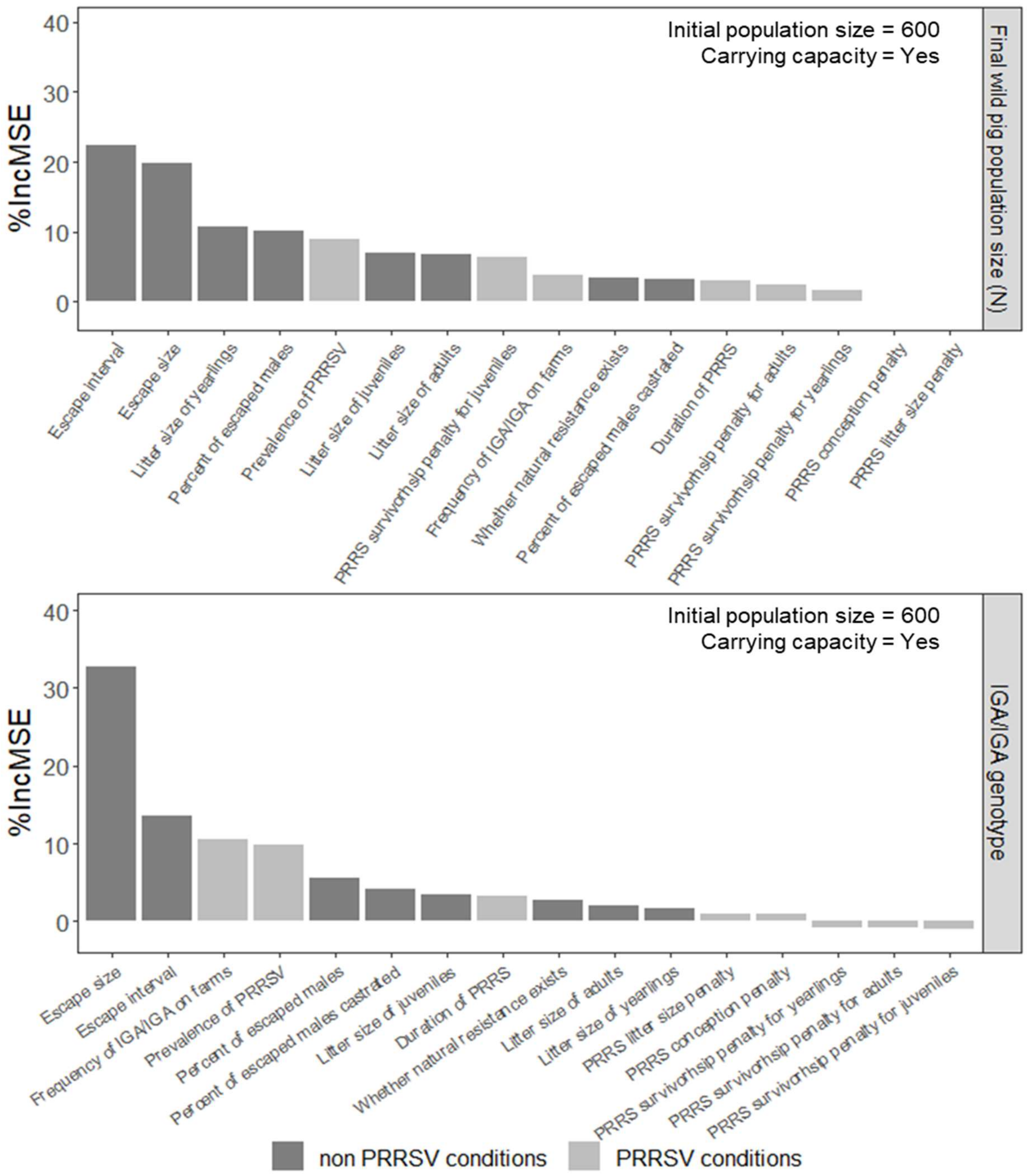

Figure A-7. Percent increase in MSE for each parameter (predictor variable) and predicted variables (N and IGA/IGA genotype) for scenarios with an initial population size of 600 and using carrying capacity. Higher values indicate greater sensitivity of predicted variables to changes in those parameters. Parameters are shaded based on whether they are parameters associated with PRRSV (PRRSV conditions) or natural, non-PRRSV related conditions (non PRRSV conditions). Note, given the low R^2 for N (Figure A-3), caution should be taken when interpreting relative importance as these parameters had essentially no power in predicting this model outcome.

Simulation of reasonable conservative and reasonable worst-case scenarios

Purpose

Simulations were run to evaluate the conclusion drawn in the EA, that the potential introduction of the pigs with the IGA into the environment would not result in anymore environmental risk than pigs without the IGA. This was achieved by simulating a broad range of reasonable conservative and reasonable worst-case scenarios to specifically evaluate the fate of the homozygous IGA/IGA genotype and any potential effects it might have on the growth of wild pig populations. Using the homozygous genotype only in the simulation modeling was most conservative because it results in resistance to PRRSV, whereas the heterozygous genotype does not.

Methods

Based on results of the sensitivity analysis, a set of reasonable conservative and reasonable worst-case scenarios were simulated to explore the effects of PRRSV on wild pig population size and frequency of IGA/IGA genotype. The scenarios were developed to explore a broad range of unlikely but possible conditions, with particular focus on those conditions that had the greatest effect on model outcomes in the sensitivity analysis. Parameter combinations explored were developed to evaluate a variety of reasonable conservative and reasonable worst-case conditions using region-specific life history traits (i.e., litter size in Texas and South Carolina) (Table A-4). These scenarios are labeled “reasonable conservative” and “reasonable worst-case” to indicate some plausibility; however, it must be emphasized many of parameter combinations evaluated are unlikely to occur. Very conservative (worst-case) scenarios were chosen to demonstrate that if a worst-case scenario would not result in greater population growth, then a more realistic scenario also would not result in greater population growth (i.e., no greater population growth, then no greater environmental harm).

Table A-4. Overview of scenarios simulated.

Wild population size (individuals)	Litter size	Carrying capacity	IGA present on farms	Escape size (individuals)	Escape interval	PRRSV prevalence (percent)
small (600)	based on Texas population	True, False	True, False	large (50)	high (yearly)	2
						5
				extremely large (600)	intermediate (every 5 years)	2
						5
large (50,000)	based on South Carolina population	True, False	True, False	large (50)	high (yearly)	2
						5
				extremely large (600)	intermediate (every 5 years)	2
						5

Litter size was an important parameter in the model based on the sensitivity analysis. Therefore, two different wild pig populations were considered for estimating this parameter, Texas and South Carolina. Texas contains many small pig farms and has a mix of areas with low and high wild pig densities, while South Carolina has many large pig farms and high wild pig density (VerCauteren et al., 2019). These sites were also chosen because studies have measured litter size statistics for the different age classes, so litter size was allowed to differ for juveniles, yearlings, and adults (Taylor et al., 1998; Chinn et al., 2022; VerCauteren et al., 2019).

The conditions of escape—size and frequency—were also often the most influential parameters in the model. Therefore, two conservative possible conditions were considered. The first set of conditions was intended to reflect a reasonable conservative case where a large number (50) of individuals escape every year into the same population. This is conservative because escapes usually include a few individuals and may not occur in the same population consistently year after year. The second set of conditions was intended to reflect a reasonable worst-case where natural disasters occur in the same population with moderate frequency, resulting in the repeated escape of an extremely large number (600) of pigs. This set of conditions is also considered a worst-case scenario because escapes of this size are very rare.

Two conditions were evaluated for wild pig population size—small and moderate-sized populations. Wild pig census population sizes vary substantially across the United States (VerCauteren et al., 2019). In order to evaluate whether the range of population sizes used in the model reflect those in the wild, wild pig population size was estimated using previously published population genetic studies. Two separate studies, one in California and one in Texas, surveyed a large number of wild pigs from across each state and employed a large number of genetic markers to estimate the spatial extent of genetically distinct populations. The geographic extent of genetically distinct populations varied tremendously between and within states, with geographic ranges varying from a single county in California to occupying over half the state of Texas (Table A-5). Using the geographic extent of these ranges and estimated pig densities for the corresponding geographic regions, census population sizes were calculated—i.e., the square miles of each region were multiplied by a range of potential wild pig densities (1-3 pigs per square mile, VerCauteren et al., 2019). Census pig population sizes were estimated to range from 737 to 391,902 pigs (Table A-5; e.g., 13,634 sq miles x 3 pigs/sq miles = 391,902 pigs), with most populations likely to be in the thousands based on the geographic extent of other genetically distinct populations (Mangan et al., 2021; Tabak et al., 2017). Thus, to encompass the broad range of the wild pig population sizes, scenarios included initial wild populations of 600 or 50,000. Simulating the lower end of population sizes was considered more critical given that small populations are expected to be more affected by the presence of the IGA (i.e., larger populations have a greater ability to reduce the likelihood that individuals are homozygous for the IGA due to dilution of many wild-type alleles in the population).

Table A-5. Estimated census wild pig population size based on genetic structure

State	Extent of genetic cluster	Square miles[1]	Estimated wild pig density (pigs/square mile)	Estimated wild pig census size (pigs)	Reference
Texas	1/2 of the state	261,268 / 2 = 130,634	1 to 3	130,616 to 391,902	Mangan et al., 2021
California	Alameda County	737	1 to 3	737 to 2,211	Tabak et al., 2017

[1] https://www.census.gov/quickfacts/fact/table/TX,alamedacountycalifornia/PST045221; accessed on July 27, 2022.

The following is an overview of the varying conditions used to develop the scenarios:

- Escapes occurring in different locations (Texas or South Carolina).

- Large escapes (50 pigs) occurring at high frequency (once per year) into the same population.

- Extremely large escapes occurring at intermediate frequency; representing natural disasters resulting in the release of 600 pigs every 5 years into the same population.

- Escapes occurring in wild populations of different sizes (small wild population of 600 or large wild population of 50,000).

- Varying prevalence of PRRSV in the wild pig population (2% or 5%).

The scenarios created from the above parameter combinations are described below. Note that four scenarios are associated with each description because each set of conditions was run with two litter size estimates (each normally distributed with a mean and standard deviation based on location (Texas and South Carolina), two age classes (juvenile yearling adult; see Table A-6) and two carrying capacity conditions (with and without) (2 x 2 = 4 variants of each scenario). Therefore, a total of 32 scenarios were simulated and are listed in Table A-6.

Scenarios 1, 9, 17, 25: A large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas/South Carolina (differing by litter size) with a small wild pig population (600 individuals) with current prevalence of PRRSV (2%).

Scenarios 2, 10, 18, 26: A large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas/South Carolina (differing by litter size) with a small wild pig population (600 individuals) with moderately higher prevalence of PRRSV (5%).

Scenarios 3, 11, 19, 27: An extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of Texas/South Carolina (differing by litter size) with a small wild pig population (600 individuals) with current prevalence of PRRSV (2%).

Scenarios 4, 12, 20, 28: An extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of Texas/South Carolina (differing by litter size) with a small wild pig population (600 individuals) with moderately higher prevalence of PRRSV (5%).

Scenarios 5, 13, 21, 29: A large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas/South Carolina (differing by litter size) with a large wild pig population (50,000 individuals) with current prevalence of PRRSV (2%).

Scenario 6, 14, 22, 30: A large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas/South Carolina (differing by litter size) with a large wild pig population (50,000 individuals) with moderately higher prevalence of PRRSV (5%).

Scenario 7, 15, 23, 31: An extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of Texas/South Carolina (differing by litter size) with a large wild pig population (50,000 individuals) with current prevalence of PRRSV (2%).

Scenario 8, 16, 24, 32: An extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of Texas/South

Carolina (differing by litter size) with a large wild pig population (50,000 individuals) with moderately higher prevalence of PRRSV (5%).

Parameters not explored in the scenarios were set to values representing reasonably conservative estimates spanning the broadest range of values observed from published studies measuring these parameters (Table A-7). The estimates are considered conservative because the effects of PRRS on survivorship, litter size, and probability of conception were based on studies from farm populations that are expected to be higher (more conservative) than what wild pigs experience. This may be due to a number of factors, for instance, PRRSV may be less virulent in the wild than on farms as a result of differing densities, stress on immune system, and levels of natural resistance that may have evolved in wild pigs (ter Beek, 2018, Wu et al., 2011).

Table A-6. Parameter values that varied for scenarios simulated; note scenarios with same values differ also by region (Texas/South Carolina) used for estimating litter size, and whether carrying capacity was implemented in the model

Scenario	Initial wild population size (pigs)	Litter size (mean)			Litter size (sd)			Carrying capacity?	Frequency of IGA/IGA on farms		Escape size (pigs)	Escape interval (years)	Prevalence of PRRSV (%)
		J	Y	A	J	Y	A		min	max			
1 (TX)	600	4.8	4.4	6.3	1.7	2.4	1.8	FALSE	0.5	1	50	1	0.02
2 (TX)	600	4.8	4.4	6.3	1.7	2.4	1.8	FALSE	0.5	1	50	1	0.05
3 (TX)	600	4.8	4.4	6.3	1.7	2.4	1.8	FALSE	0.5	1	600	5	0.02
4 (TX)	600	4.8	4.4	6.3	1.7	2.4	1.8	FALSE	0.5	1	600	5	0.05
5 (TX)	50,000	4.8	4.4	6.3	1.7	2.4	1.8	FALSE	0.5	1	50	1	0.02
6 (TX)	50,000	4.8	4.4	6.3	1.7	2.4	1.8	FALSE	0.5	1	50	1	0.05
7 (TX)	50,000	4.8	4.4	6.3	1.7	2.4	1.8	FALSE	0.5	1	600	5	0.02
8 (TX)	50,000	4.8	4.4	6.3	1.7	2.4	1.8	FALSE	0.5	1	600	5	0.05
9 (TX)	600	4.8	4.4	6.3	1.7	2.4	1.8	TRUE	0.5	1	50	1	0.02
10 (TX)	600	4.8	4.4	6.3	1.7	2.4	1.8	TRUE	0.5	1	50	1	0.05
11 (TX)	600	4.8	4.4	6.3	1.7	2.4	1.8	TRUE	0.5	1	600	5	0.02
12 (TX)	600	4.8	4.4	6.3	1.7	2.4	1.8	TRUE	0.5	1	600	5	0.05
13 (TX)	50,000	4.8	4.4	6.3	1.7	2.4	1.8	TRUE	0.5	1	50	1	0.02
14 (TX)	50,000	4.8	4.4	6.3	1.7	2.4	1.8	TRUE	0.5	1	50	1	0.05
15 (TX)	50,000	4.8	4.4	6.3	1.7	2.4	1.8	TRUE	0.5	1	600	5	0.02
16 (TX)	50,000	4.8	4.4	6.3	1.7	2.4	1.8	TRUE	0.5	1	600	5	0.05
17 (SC)	600	4.3	5.5	5.9	3.3	3.2	2.8	TRUE	0.5	1	50	1	0.02
18 (SC)	600	4.3	5.5	5.9	3.3	3.2	2.8	TRUE	0.5	1	50	1	0.05
19 (SC)	600	4.3	5.5	5.9	3.3	3.2	2.8	TRUE	0.5	1	600	5	0.02
20 (SC)	600	4.3	5.5	5.9	3.3	3.2	2.8	TRUE	0.5	1	600	5	0.05
21 (SC)	50,000	4.3	5.5	5.9	3.3	3.2	2.8	TRUE	0.5	1	50	1	0.02
22 (SC)	50,000	4.3	5.5	5.9	3.3	3.2	2.8	TRUE	0.5	1	50	1	0.05
23 (SC)	50,000	4.3	5.5	5.9	3.3	3.2	2.8	TRUE	0.5	1	600	5	0.02
24 (SC)	50,000	4.3	5.5	5.9	3.3	3.2	2.8	TRUE	0.5	1	600	5	0.05

25 (SC)	600	4.3	5.5	5.9	3.3	3.2	2.8	FALSE	0.5	1	50	1	0.02
26 (SC)	600	4.3	5.5	5.9	3.3	3.2	2.8	FALSE	0.5	1	50	1	0.05
27 (SC)	600	4.3	5.5	5.9	3.3	3.2	2.8	FALSE	0.5	1	600	5	0.02
28 (SC)	600	4.3	5.5	5.9	3.3	3.2	2.8	FALSE	0.5	1	600	5	0.05
29 (SC)	50,000	4.3	5.5	5.9	3.3	3.2	2.8	FALSE	0.5	1	50	1	0.02
30 (SC)	50,000	4.3	5.5	5.9	3.3	3.2	2.8	FALSE	0.5	1	50	1	0.05
31 (SC)	50,000	4.3	5.5	5.9	3.3	3.2	2.8	FALSE	0.5	1	600	5	0.02
32 (SC)	50,000	4.3	5.5	5.9	3.3	3.2	2.8	FALSE	0.5	1	600	5	0.05

Table A-7. Parameter values not varied for scenarios simulated; parameters not listed were set to those found in Table A-2

Parameter	Units	Distribution	Values for simulation
Percent of escaped males	percent	uniform	min = 0, max = 1
Percent of escaped males castrated	percent	uniform	min = 0, max = 1
Maximum age of wild pigs	years	uniform	min = 8, max = 14
Age of farm animals	years	uniform	min =1; max = 14
Duration of PRRS	months	uniform	min = 1.5, max = 8
Whether natural resistance exists	-	-	FALSE
Age at sexual maturity	months	uniform	min = 3, max = 12
Gestation period	months	uniform	min = 6, max = 12
Conception rate	percent	uniform	min = 0.12, max = 1
PRRS conception penalty	percent	uniform	min = 0, max = 0.5
PRRS litter size penalty	percent	uniform	min 0.1, max = 1
PRRS survivorship penalty for juveniles	percent	uniform	min = 0, max = 0.2
PRRS survivorship penalty for yearlings	percent	uniform	min = 0, max = 0.1
PRRS survivorship penalty for adults	percent	uniform	min = 0, max = 0.1
Background/Natural mortality	percent	normal	mean = 0.56, sd = 0.26

Additional scenarios were simulated to act as “controls” for determining whether the fate and effects of the IGA differ from the status quo. That is, to compare the fate (frequency) of the IGA to what would be expected for natural genetic variation in domestic pigs that has no fitness consequences (i.e., neutral genetic variation), additional scenarios were run with the prevalence of PRRSV set to zero, effectively making the IGA neutral (i.e., with no PRRSV in the environment, resistance to PRRSV provides no fitness advantage). This control represents a scenario where a mutation (like the IGA or any other genetic variation) evolves neutrally, where it is affected by all factors (e.g., demographics processes and migration) with the exception of natural selection. To distinguish the effects of escaped domestic pigs that do not contain the IGA to those that do, additional scenarios were simulated where pigs escaping from the farm population contained no IGA alleles. This scenario represents what would happen if there were an escape from a farm that did not house pigs that contain the IGA. Any difference between these outcomes would reflect the effect of the IGA. To statistically assess any such differences, an unpaired t-test was used to compare the final population size (last month in simulation) of each scenario to its respective control. Although both the fate of the IGA allele and the PRRSV-resistant phenotype (IGA/IGA genotype) could be measured, the PRRSV-resistant phenotype is the primary exposure of concern given that it has the potential to confer a fitness advantage, whereas measuring the frequency of the IGA allele would include heterozygous individuals that are not phenotypically different from domestic pigs that do not contain the IGA.

All scenarios were simulated 1,000 times, and each scenario used the same set of randomly selected seeds to ensure consistency among scenarios.[24] The scenarios simulated a 20-60-year period. As was performed in the sensitivity analysis, for parameter combinations where carrying capacity was used, the simulations were run for a total of 60 years (10-year burn-in), while for scenarios where there was no carrying capacity, the simulation was run for a total of 20 years (10-year burn-in) due to the larger computational demand that arises when the population is allowed to grow unchecked. The first 10 years of every scenario were used as a burn-in (discarded) to ensure that the population size and demographics stabilized. Escape from farm populations began following the burn-in period.

It is worth noting that the fitness advantage (via effects on survivorship, litter size, and probability of conception) as implemented in the model is expected to be more conservative (i.e., greater effects) than would be experienced in the wild for several reasons. First, the prevalence of PRRSV is fixed at a constant frequency every generation. In reality, as the frequency of resistant individuals (homozygous for the IGA) increases, the prevalence of PRRSV would be expected to decrease via negative feedback, which would in turn lower the relative fitness benefit of being resistant (i.e., being homozygous for the IGA). Second, the reproductive fitness costs assigned to individuals lacking the IGA (not resistant to PRRSV) is expected to be an overestimate given that the health effects of PRRS are based on observations from farm populations (as discussed previously). Further, the model was run with no natural immunity to PRRSV, even short-term immunity (1 year), or reduced fitness effects for those that previously contracted the disease, as only an IGA-mediated resistance/immunity existed in this model. In reality, some level of natural immunity may exist (ter Beek, 2018; Wu et al., 2011) or could emerge and would reduce the relative fitness benefit of having the IGA-mediated, PRRSV-resistant phenotype. Thus, the IGA is expected to confer a greater fitness advantage in individuals in the model than would be expected under real-world conditions.

Results

Whether litter size estimates were based on Texas or South Carolina wild pig populations, the results were very similar quantitatively (i.e., in terms of statistical significance) and identical qualitatively (i.e., the trends of the data). Results are presented by combining sets of scenarios that have the same conditions but include differences in prevalence of PRRSV (2% and 5%) (e.g., Scenarios 1 and 2). Results are presented first for Texas, then South Carolina, and for each set of scenarios, results are provided first for conditions with no carrying capacity and then for conditions with carrying capacity. When the trajectories overlap, the lines on the graphs appear dotted, i.e., there is no difference in the lines for each scenario in the graph (see Figures A-8, A-9 a and b, A-11 b, A-13 a and b, A-14 a, A-15 a and b, A-16 a, A-17, A-18 a and b, A-20 b, A-22 a and b, A-23 a, A-24 a and b, A-25 a).

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[24] The seed value determines how random numbers are generated. Using the same set of seeds ensures that this randomization is consistent across scenarios allowing for comparisons among scenarios, as model outputs can be more reliably attributed to the specific methods in use, rather than the unpredictable nature of random number generation.

Texas population

Populations stabilized prior to the completion of the burn-in period for all scenarios, that is, all scenarios demonstrated the same trend (Figure A-8; representative of all scenarios for this population).

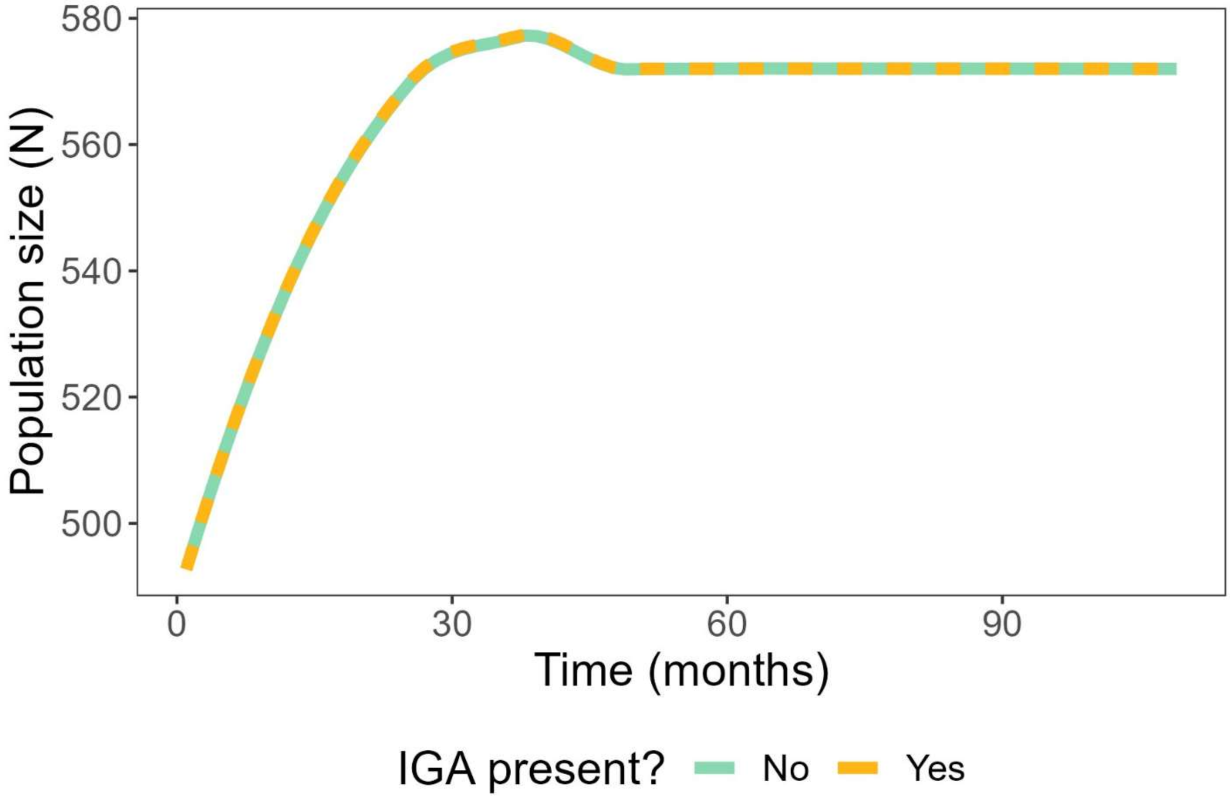

Figure A-8. Population size during burn-in (first 10 years) demonstrating populations stabilizing prior to escape events (months > 120). The population size is the same in both scenarios, so the trajectories overlap.

Scenarios 1 and 2 (without carrying capacity), and Scenarios 9 and 10 (with carrying capacity) – a large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas with a small wild pig population (600 individuals) with current and moderately higher prevalence of PRRSV (2 and 5%, respectively).

<u>Scenarios without carrying capacity</u>

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation, regardless of the prevalence of PRRSV (2% or 5%)—the IGA/IGA genotype stayed at low frequency and began to decline over the 10-year period simulated (Figure A-9a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size grew as expected over time (Figures A-9b, A-9c). Populations grew slower when prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

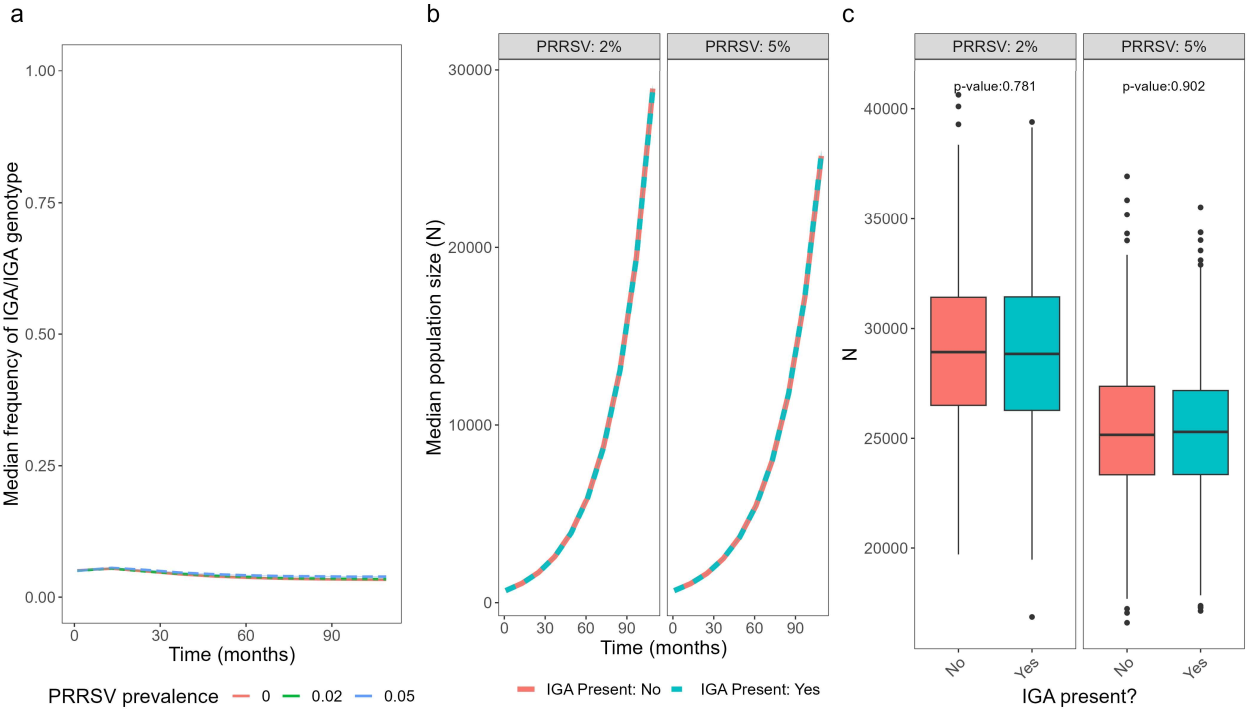

Figure A-9. **Fate of IGA/IGA genotype and its effects on wild pig populations with no carrying capacity. Simulations consisted of a large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas with a small wild pig population (600 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

Scenarios with carrying capacity

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation—the IGA/IGA genotype increased in frequency over time but appeared to plateau prior to the end of the simulation (~400-600 months) (Figure A-10a). However, after ~10 years, the rate of increase of the IGA/IGA genotype was positively correlated with increasing prevalence of PRRSV, with higher prevalence resulting in higher frequency of the IGA/IGA genotype.

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size remained relatively stable over time (Figures A-10b, A-10c).

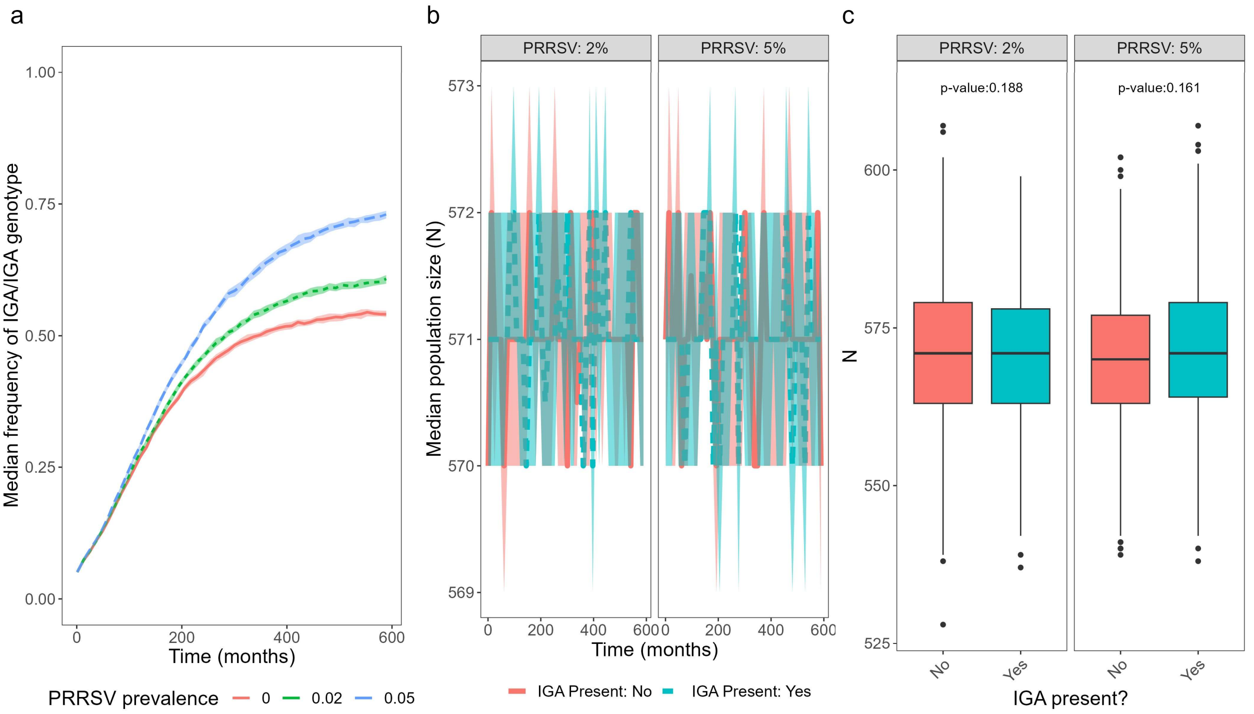

Figure A-10. **Fate of IGA/IGA genotype and its effects on wild pig populations with carrying capacity. Simulations consisted of a large number (50 individuals) of escapes occurring regularly (once per year) in a part of Texas with a small wild pig population (600 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

In summary, if a large number of pigs containing the IGA were to regularly escape in a location similar to Texas with a small wild pig population, the frequency of the IGA/IGA genotype may increase to a higher frequency than neutral genetic variation in stable populations, particularly as the prevalence of PRRSV increases. However, the presence of the IGA/IGA genotype would be expected to have no effect on wild pig population size since the differences are not statistically significant (see Figures A-9 and A-10 b and c).

Scenarios 3 and 4 (without carrying capacity), and Scenarios 11 and 12 (with carrying capacity) – an extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of Texas with a small wild pig population (600 individuals) with current and moderately higher prevalence of PRRSV (2 and 5%, respectively).

<u>Scenarios without carrying capacity</u>

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation—the IGA/IGA increased with each escape event, particularly the first event, but began to decline following escape (Figure A-11a). After the second escape event, the frequency of the IGA/IGA genotype was slightly higher when the prevalence of PRRSV was 5%, but only by 2.6%.

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size grew as expected over time (Figures A-11b, A-11c). Populations grew slightly slower when the prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

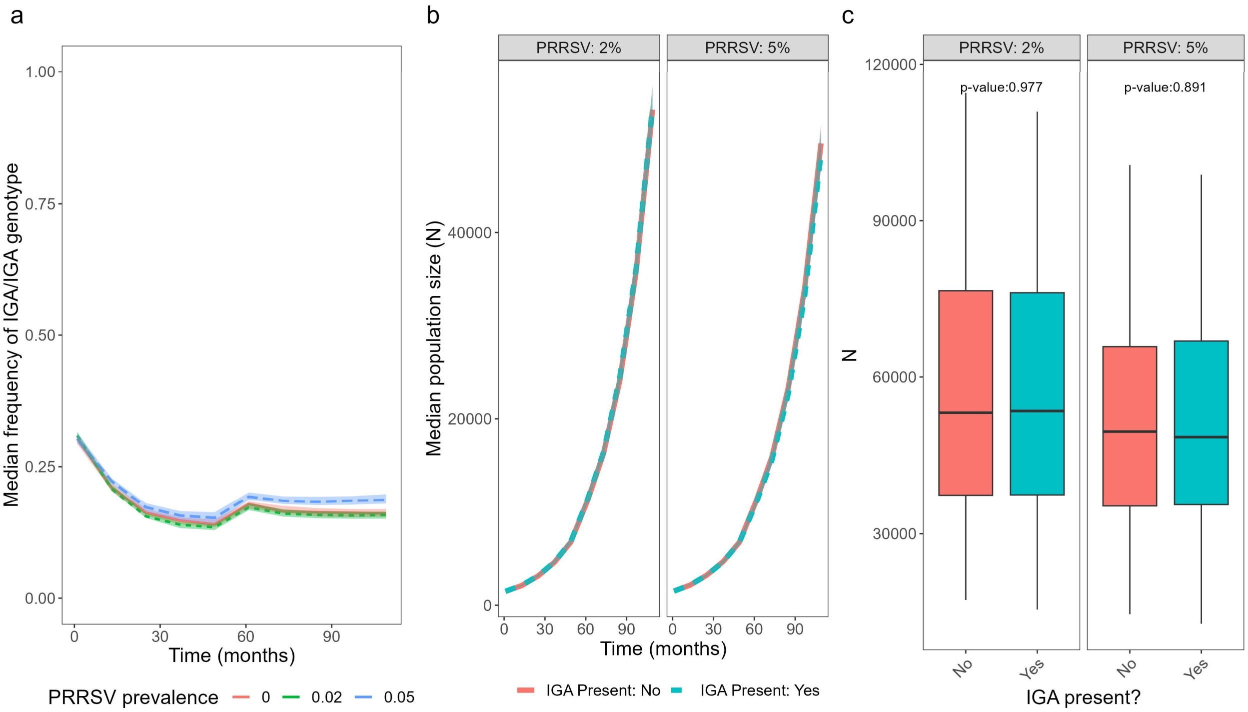

Figure A-11. Fate of IGA/IGA genotype and its effects on wild pig populations with no carrying capacity. Simulations consisted of an extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of Texas with a small wild pig population (600 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).

<u>Scenarios with carrying capacity</u>

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation—the IGA/IGA genotype increased in frequency with each escape event, but appeared to plateau prior to the end of the simulation (~200-600 months) (Figure A-12a). However, after ~10 years, the rate of increase of the IGA/IGA genotype was positively correlated with increasing prevalence of PRRSV, with higher prevalence resulting in higher frequency of the IGA/IGA genotype.

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size remained relatively stable over time (Figure A-12b, A-12c).

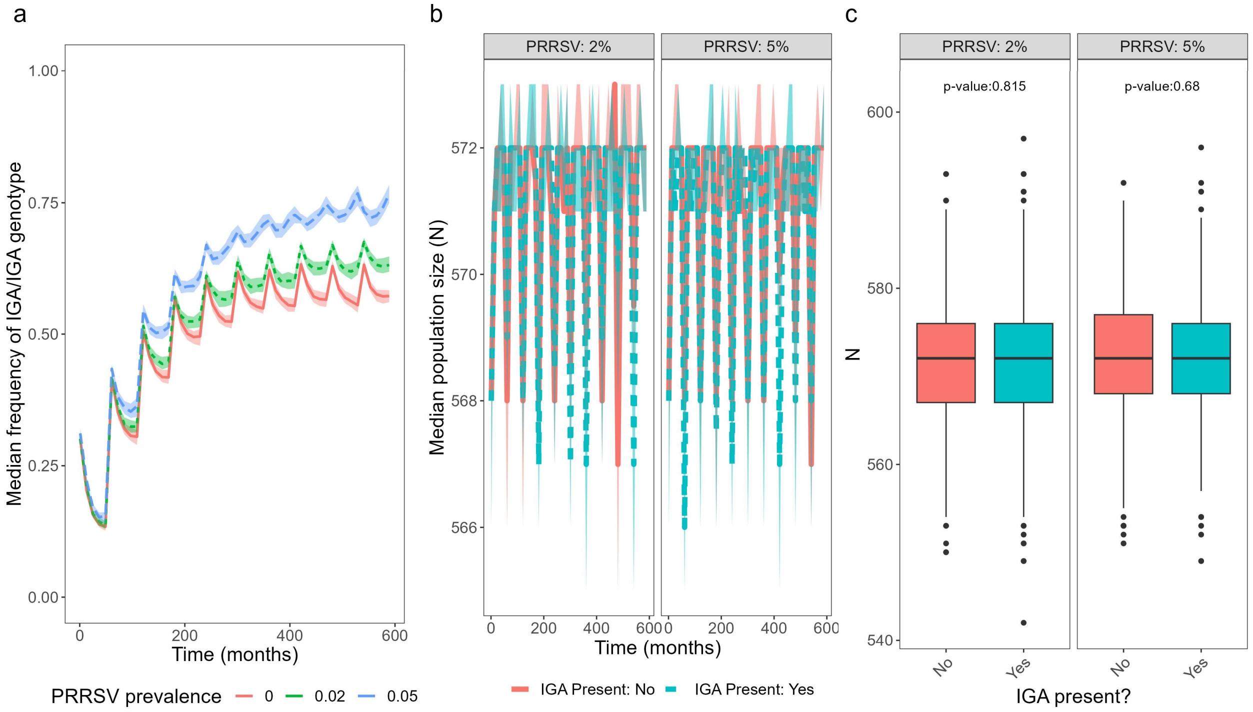

Figure A-12. Fate of IGA/IGA genotype and its effects on wild pig populations with carrying capacity. Simulations consisted of an extremely large number of escapes (600 individuals) occurring at an intermediate frequency in a part of Texas with a small wild pig population (once every 5 years) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).

In summary, if an extremely large number of pigs containing the IGA were to escape with an intermediate frequency (e.g., as a result of a natural disaster) in a location similar to Texas with a small wild pig population, the frequency of the IGA/IGA genotype may increase to a higher frequency than neutral genetic variation in stable populations, particularly as the prevalence of PRRSV increases. However, the presence of the IGA/IGA genotype would be expected to have no effect on wild pig population size since the differences are not statistically significant (see Figures A-11 and A-12 b and c).

Scenarios 5 and 6 (without carrying capacity), and Scenarios 13 and 14 (with carrying capacity) – a large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas with a large wild pig population (50,000 individuals) with current and moderately higher prevalence of PRRSV (2 and 5%, respectively).

<u>Scenarios without carrying capacity</u>

The frequency of the IGA/IGA genotype behaved almost identically to neutral genetic variation, regardless of the prevalence of PRRSV (2% or 5%)—the IGA/IGA genotype stayed at near zero frequency over the 10-year period simulated (Figure A-13a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size grew as expected over time (Figure A-

13b, A-13c). Populations grew slower when the prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

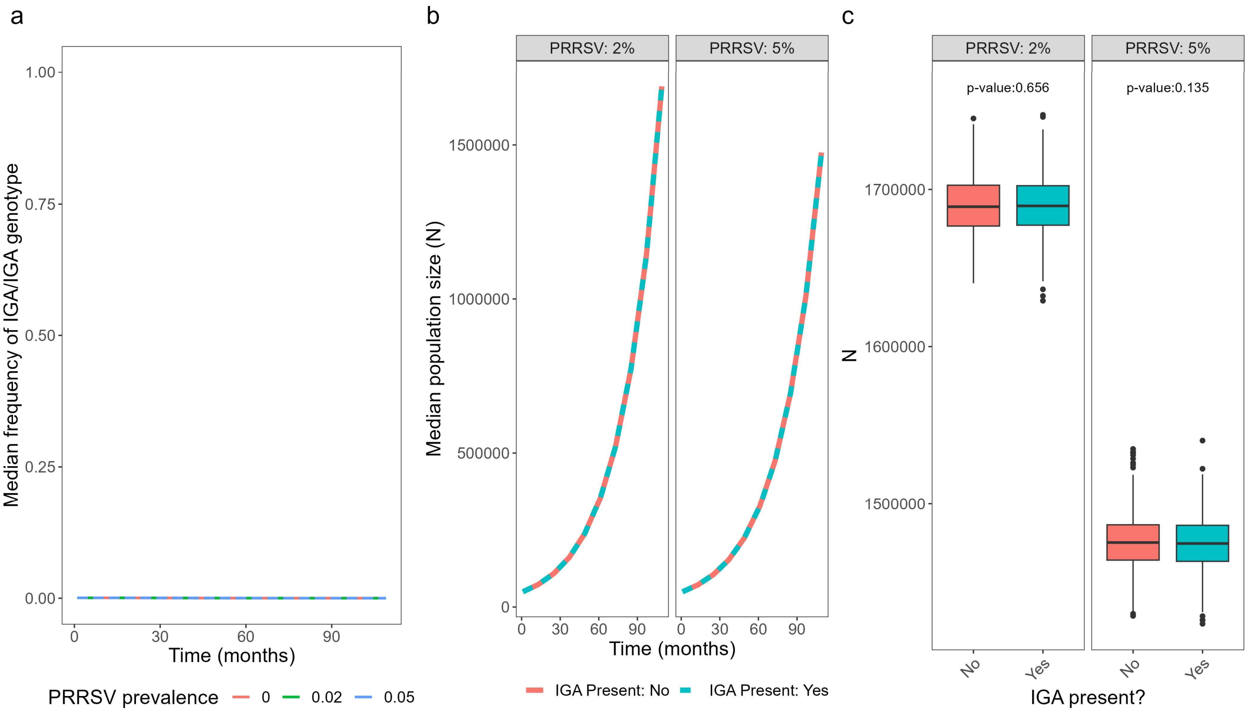

Figure A-13. **Fate of IGA/IGA genotype and its effects on wild pig populations with no carrying capacity. Simulations consisted of a large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas with a large wild pig population (50,000 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

Scenarios with carrying capacity

The frequency of the IGA/IGA genotype behaved almost identically to neutral genetic variation, regardless of the prevalence of PRRSV (2% or 5%)—the IGA/IGA genotype stayed at near zero frequency over the 50-year period simulated (Figure A-14a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size remained relatively stable over time (Figures A-14b, A-14c). Populations stabilized at a lower population size when the prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

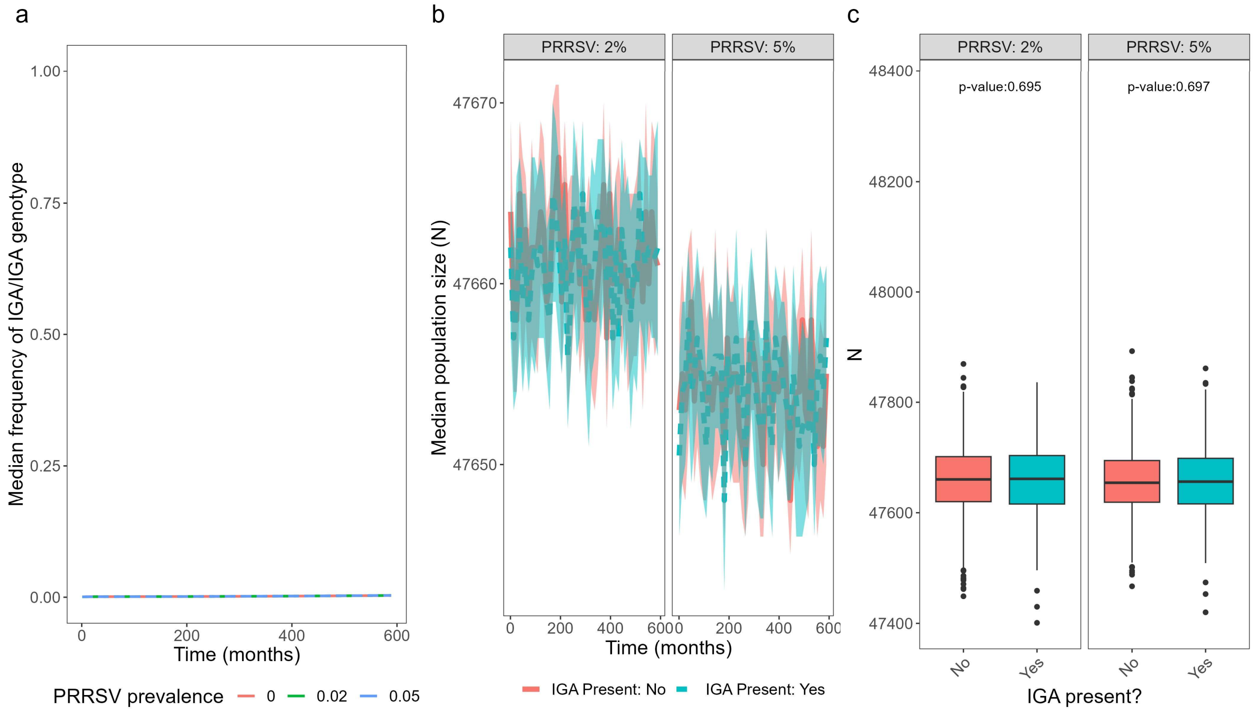

Figure A-14. **Fate of IGA/IGA genotype and its effects on wild pig populations with carrying capacity. Simulations consisted of a large number of escapes (50 individuals) occurring regularly (once per year) in a part of Texas with a large wild pig population (50,000 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

In summary, if a large number of pigs containing the IGA were to regularly escape in a location similar to Texas with a large wild pig population, the frequency of the IGA/IGA genotype would remain at extremely low frequency (near zero). Further, the presence of the IGA/IGA genotype would be expected to have no effect on wild pig population size since the differences are not statistically significant (see Figures A-13 and A-14 b and c).

Scenarios 7 and 8 (without carrying capacity), and Scenarios 15 and 16 (with carrying capacity) – an extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of Texas with a large wild pig population (50,000 individuals) with current and moderately higher prevalence of PRRSV (2 and 5%, respectively).

Scenarios without carrying capacity

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation, regardless of the prevalence of PRRSV (2% or 5%)—the IGA/IGA genotype stayed at an extremely low frequency (near zero) (Figure A-15a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size grew as expected over time (Figures A-15b, A-15c). Populations grew slower when the prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

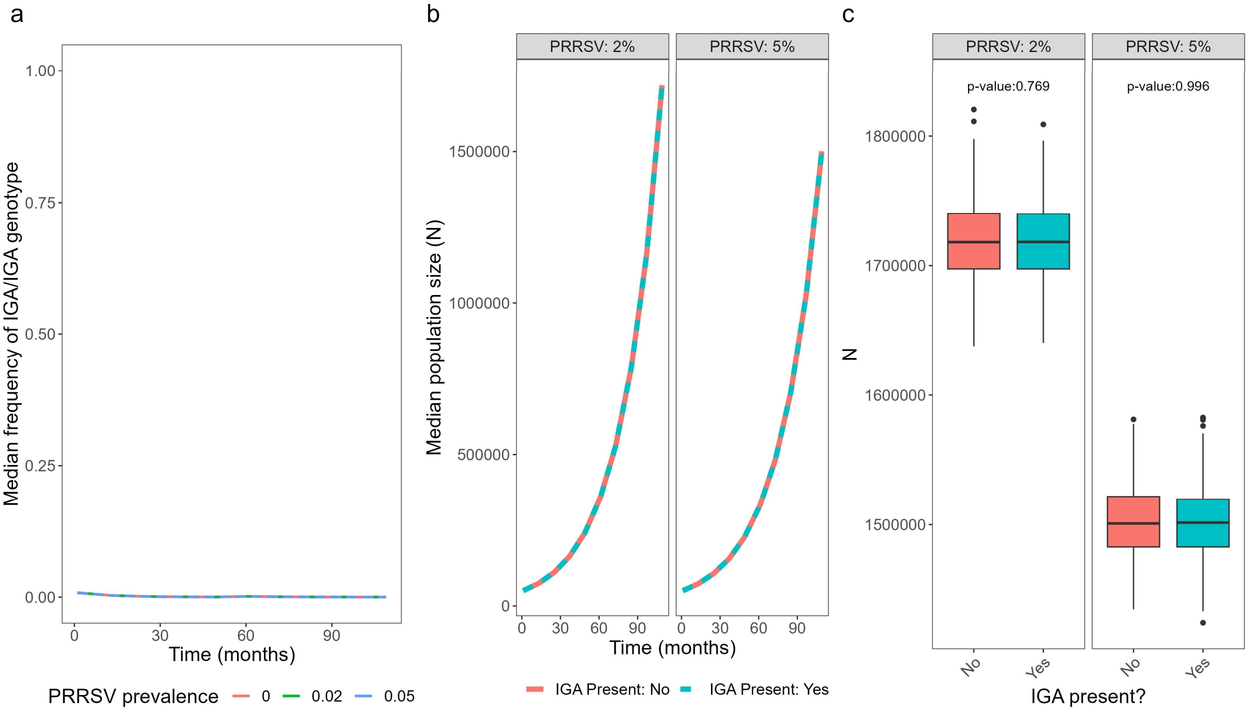

Figure A-15. **Fate of IGA/IGA genotype and its effects on wild pig populations with no carrying capacity. Simulations consisted of an extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of Texas with a large wild pig population (50,000 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

Scenarios with carrying capacity

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation—the IGA/IGA genotype increased slightly with every escape event but remained at an extremely low frequency (near zero) over the entire 50-year period (Figure A-16a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size remained relatively stable over time (Figures A-16b, A-16c). Populations stabilized at a lower population size when the prevalence of PRRSV was greater (5% vs. 2%), but did not differ based on the presence of the IGA.

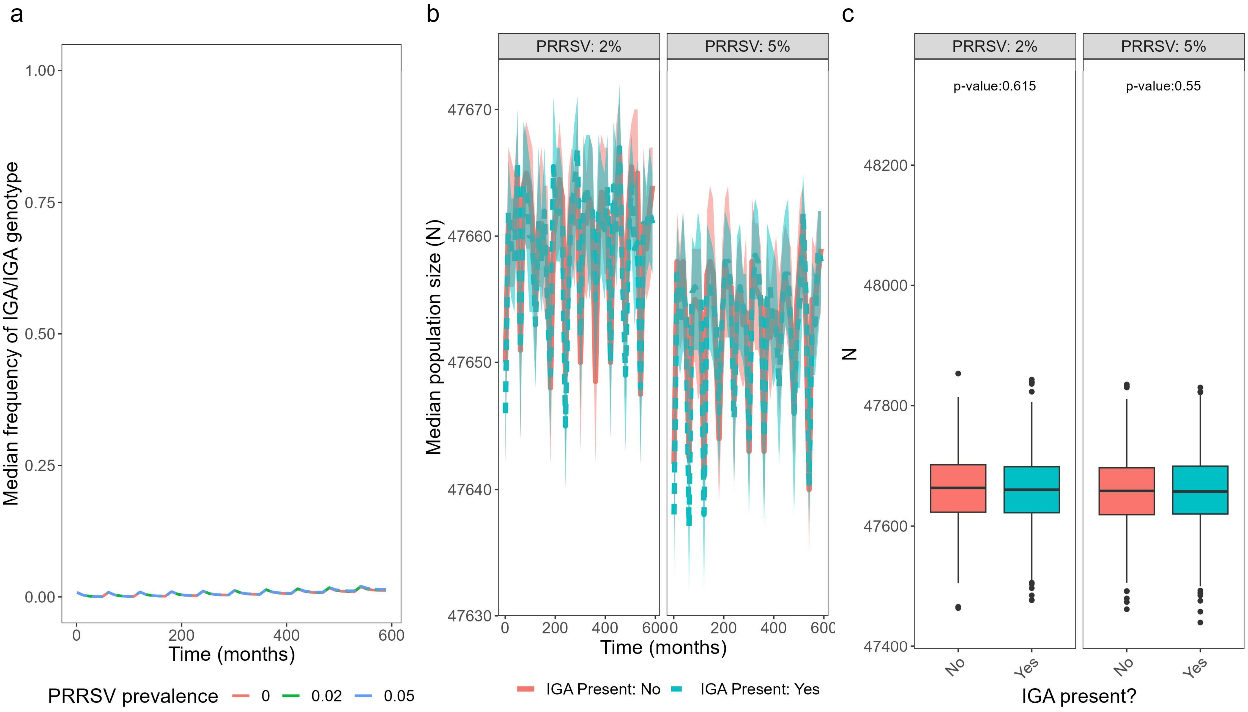

Figure A-16. **Fate of IGA/IGA genotype and its effects on wild pig populations with carrying capacity. Simulations consisted of an extremely large number of escapes (600 individuals) occurring at an intermediate frequency (once every 5 years) in a part of Texas with a large wild pig population (50,000 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

In summary, if an extremely large number of pigs containing the IGA were to escape with an intermediate frequency (e.g., as a result of a natural disaster) in a location similar to Texas with a large wild pig population, the frequency of the IGA/IGA genotype would be expected to remain at an extremely low frequency (near zero). Further, the presence of the IGA/IGA genotype would be expected to have no effect on wild pig population size since the differences are not statistically significant (see Figures A-15 and A-16 b and c).

South Carolina population

Populations stabilized prior to the completion of the burn-in period for all scenarios, that is, all scenarios demonstrated the same trend (Figure A-17; representative of all scenarios for this population).

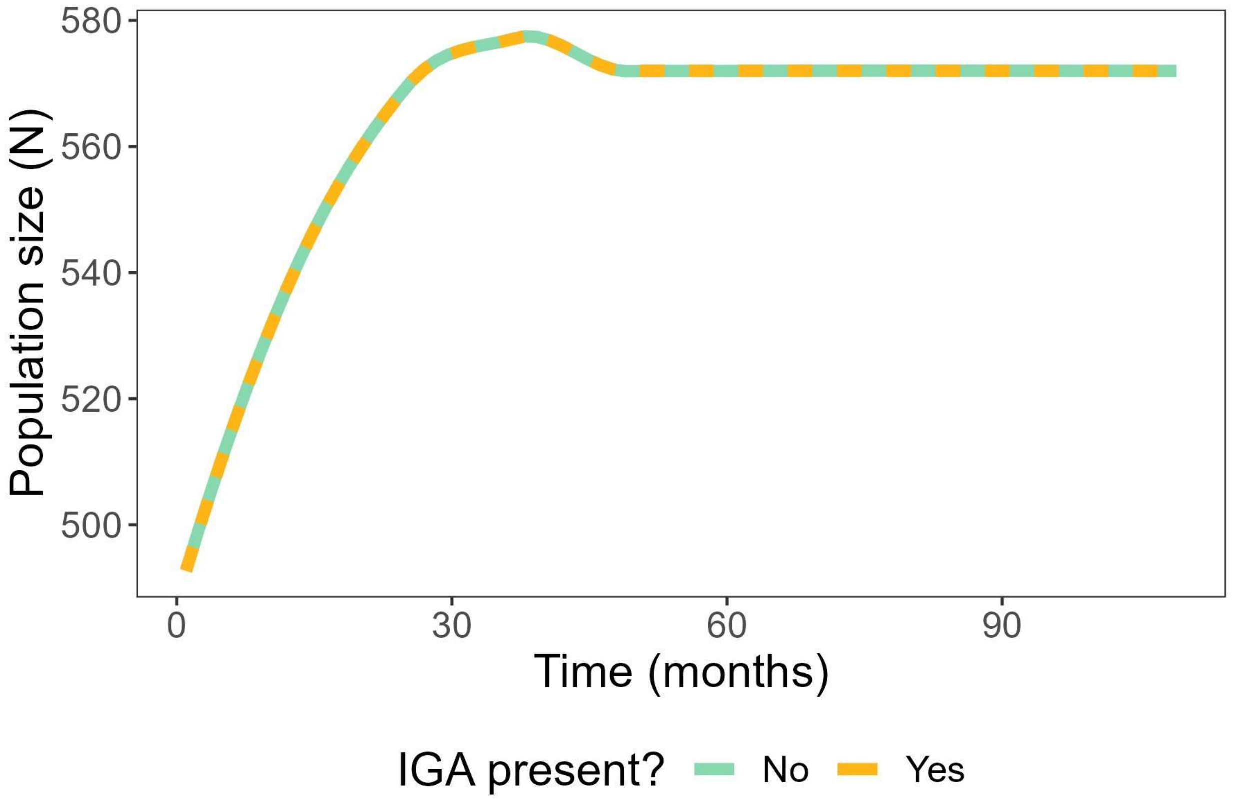

Figure A-17. **Population size during burn-in (first 10 years) demonstrating populations stabilizing prior to escape events (months > 120). The population size is the same in both scenarios, so the trajectories overlap.**

Scenarios 17 and 18 (without carrying capacity), and Scenarios 25 and 26 (with carrying capacity) – a large number of escapes (50 individuals) occurring regularly (once per year) in a part of South Carolina with a small wild pig population (600 individuals) with current and moderately higher prevalence of PRRSV (2 and 5%, respectively).

<u>Scenarios without carrying capacity</u>

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation, regardless of the prevalence of PRRSV (2% or 5%)—the IGA/IGA genotype stayed at low frequency and began to decline over the 10-year period simulated (Figure A-18a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size grew as expected over time (Figures A-18b, A-18c). Populations grew slower when prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

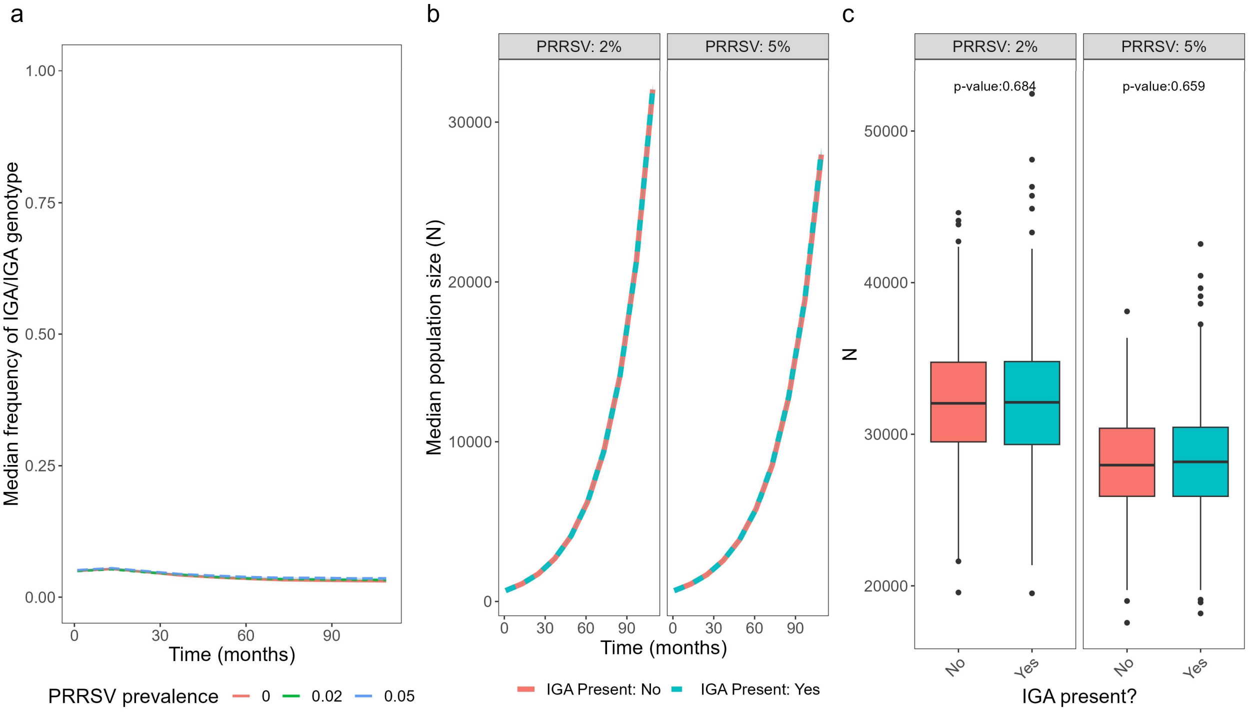

Figure A-18. **Fate of IGA/IGA genotype and its effects on wild pig populations with no carrying capacity. Simulations consisted of a large number of escapes (50 individuals) occurring regularly (once per year) in a part of South Carolina with a small wild pig population (600 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

Scenarios with carrying capacity

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation—the IGA/IGA genotype increased in frequency over time but appeared to plateau prior to the end of the simulation (~400-600 months) (Figure A-19a). However, after ~10 years, the rate of increase of the IGA/IGA genotype was positively correlated with increasing prevalence of PRRSV, with higher prevalence resulting in higher frequency of the IGA/IGA genotype.

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size remained relatively stable over time (Figures A-19b, A-19c).

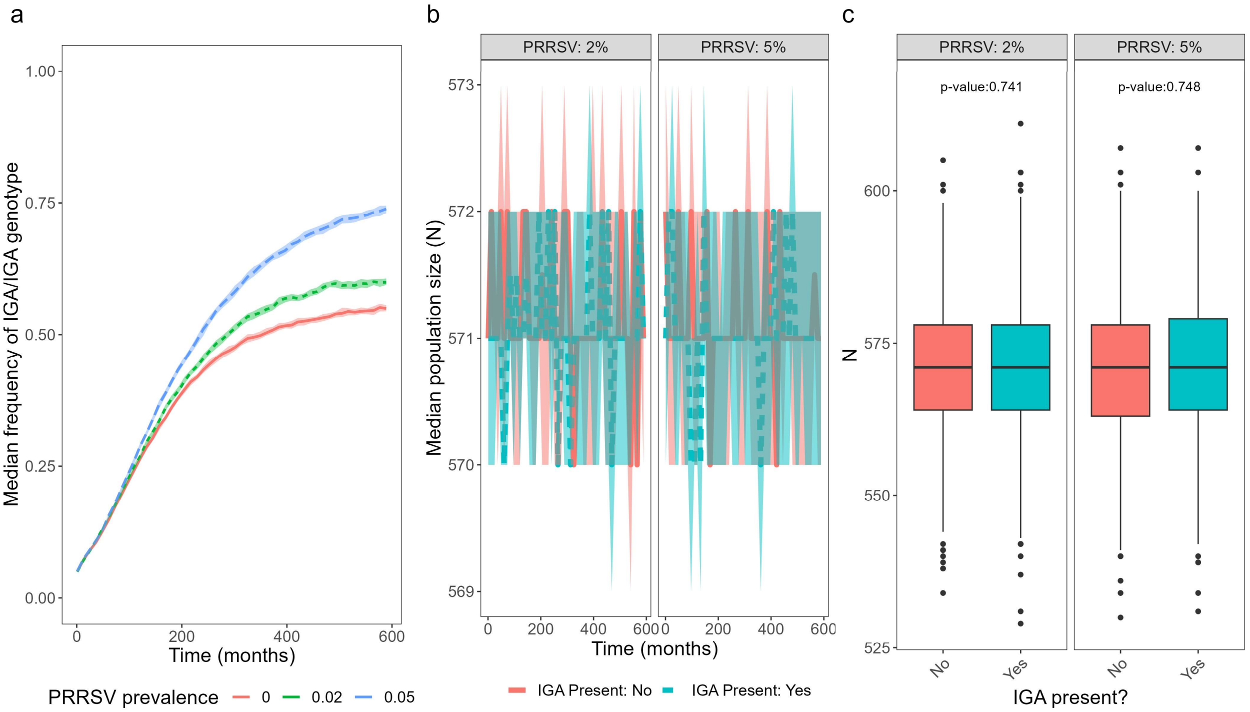

Figure A-19. **Fate of IGA/IGA genotype and its effects on wild pig populations with carrying capacity. Simulations consisted of a large number of escapes (50 individuals) occurring regularly (once per year) in a part of South Carolina with a small wild pig population (600 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

In summary, if a large number of pigs containing the IGA were to regularly escape in a location similar to South Carolina with a small wild pig population, the frequency of the IGA/IGA genotype may increase to a higher frequency than neutral genetic variation in stable populations, particularly as the prevalence of PRRSV increases. However, the presence of the IGA/IGA genotype would be expected to have no effect on wild pig population size since the differences are not statistically significant (see Figures A-18 and A-19 b and c).

Scenarios 19 and20 (without carrying capacity), and Scenarios 27 and 28 (with carrying capacity) – an extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of South Carolina with a small wild pig population (600 individuals) with current and moderately higher prevalence of PRRSV (2 and 5%, respectively).

<u>Scenarios without carrying capacity</u>

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation—the IGA/IGA genotype increased with each escape event, particularly the first event, but began to decline following escape (Figure A-20a). After the second escape event, the frequency of the IGA/IGA genotype was slightly higher when the prevalence of PRRSV was 5%, but only by 3.1%.

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size grew as expected over time (Figures A-20b, A-20c). Populations grew slightly slower when the prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

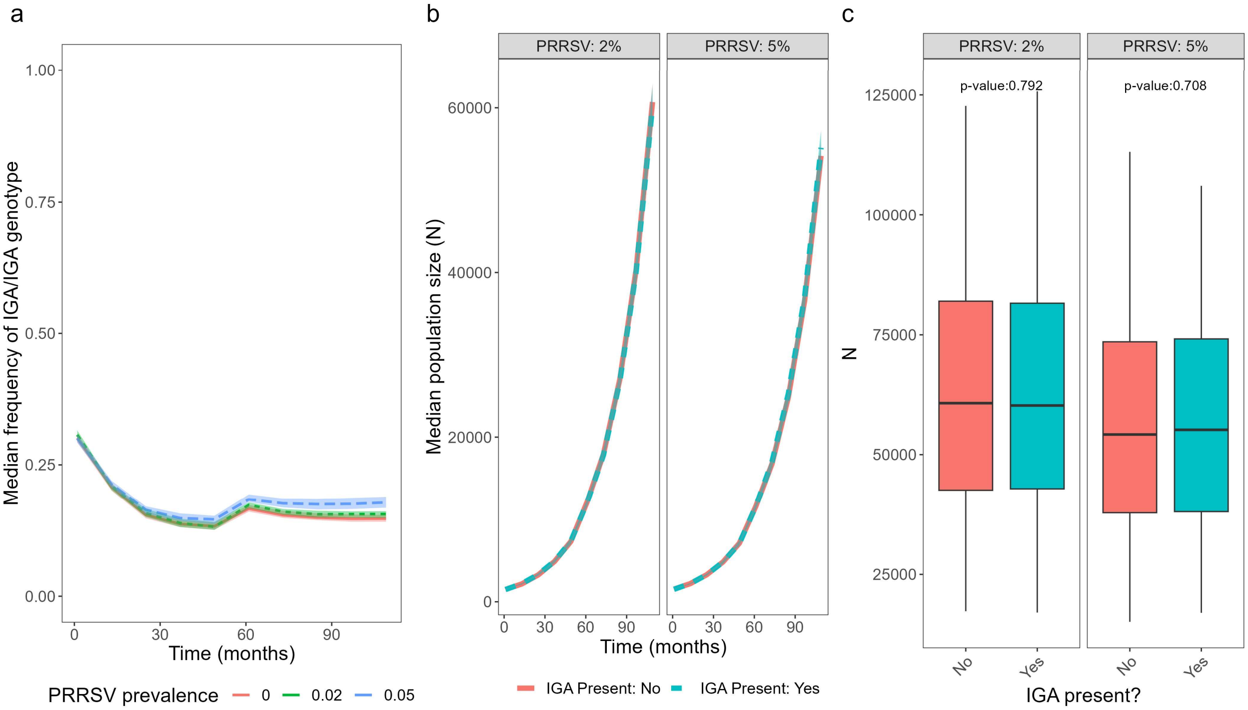

Figure A-20. **Fate of IGA/IGA genotype and its effects on wild pig populations with no carrying capacity. Simulations consisted of an extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of South Carolina with a small wild pig population (600 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

<u>Scenarios with carrying capacity</u>

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation—the IGA/IGA genotype increased in frequency with each escape event. but appeared to plateau prior to the end of the simulation (~200-600 months) (Figure A-21a). However, after ~10 years, the rate of increase of the IGA/IGA genotype was positively correlated with increasing prevalence of PRRSV, with higher prevalence resulting in higher frequency of the IGA/IGA genotype.

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size remained relatively stable over time (Figure A-21b, A-21c).

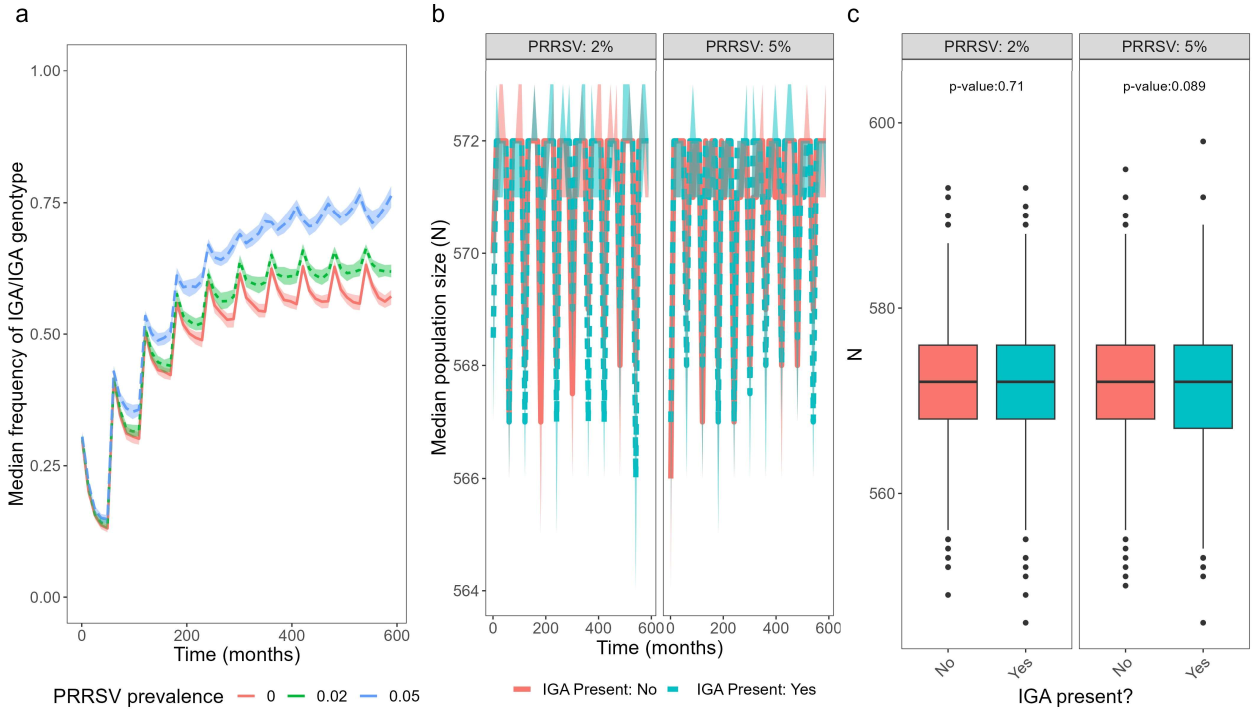

Figure A-21. Fate of IGA/IGA genotype and its effects on wild pig populations with carrying capacity. Simulations consisted of an extremely large number of escapes (600 individuals) occurring at an intermediate frequency (once every 5 years) in a part of South Carolina with a small wild pig population (600 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).

In summary, if an extremely large number of pigs containing the IGA were to escape with an intermediate frequency (e.g., as a result of a natural disaster) in a location similar to South Carolina with a small wild pig population, the frequency of the IGA/IGA genotype may increase to a higher frequency than neutral genetic variation in stable populations, particularly as the prevalence of PRRSV increases. However, the presence of the IGA/IGA genotype would be expected to have no effect on wild pig population size since the differences are not statistically significant (see Figures A-20 and A-21 b and c).

Scenarios 21 and 22 (without carrying capacity), and Scenarios 29 and 30 (with carrying capacity) – a large number of escapes (50 individuals) occurring regularly (once a year) in a part of South Carolina with a large wild pig population (50,000 individuals) with current and moderately higher prevalence of PRRSV (2 and 5%, respectively).

<u>Scenarios without carrying capacity</u>

The frequency of the IGA/IGA genotype behaved almost identically to neutral genetic variation, regardless of the prevalence of PRRSV (2% or 5%)—the IGA/IGA genotype stayed at near zero frequency over the 10-year period simulated (Figure A-22a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size grew as expected over time (Figure A-

22b, A-22c). Populations grew slower when the prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

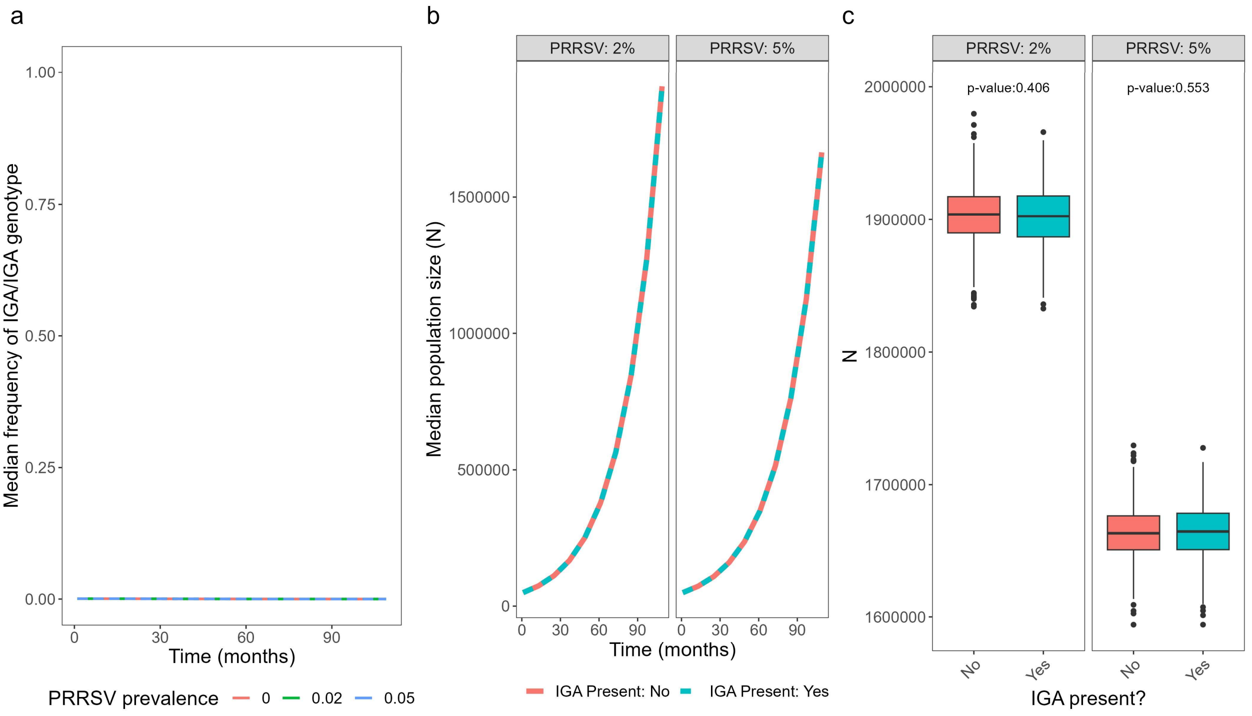

Figure A-22. **Fate of IGA/IGA genotype and its effects on wild pig populations with no carrying capacity. Simulations consisted of a large number of escapes (50 individuals) occurring regularly (once per year) in a part of South Carolina with a large wild pig population (50,000 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

Scenarios with carrying capacity

The frequency of the IGA/IGA genotype behaved almost identically to neutral genetic variation, regardless of the prevalence of PRRSV (2% or 5%)—the IGA/IGA genotype stayed at near zero frequency over the 50-year period simulated (Figure A-23a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size remained relatively stable over time (Figures A-23b, A-23c). Populations stabilized at a lower population size when the prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

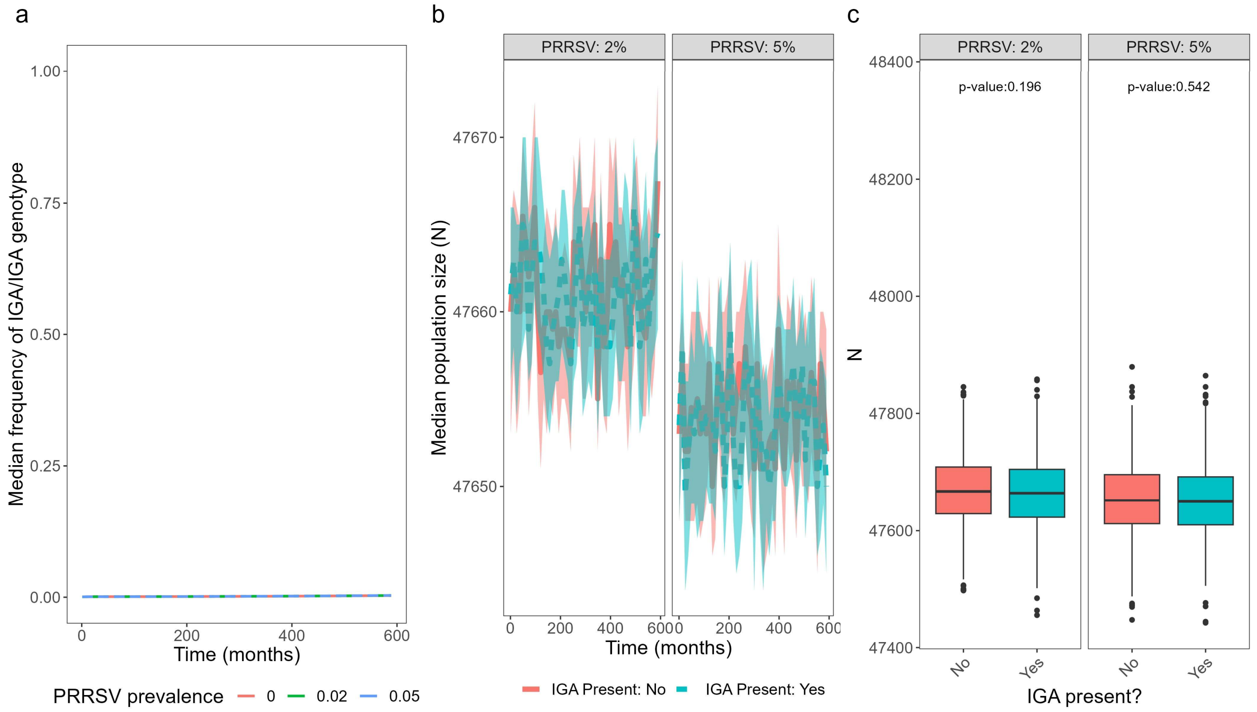

Figure A-23. **Fate of IGA/IGA genotype and its effects on wild pig populations with carrying capacity. Simulations consisted of a large number of escapes (50 individuals) occurring regularly (once per year) in a part of South Carolina with a large wild pig population (50,000 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

In summary, if a large number of pigs containing the IGA were to regularly escape in a location similar to South Carolina with a large wild pig population, the frequency of the IGA/IGA genotype would remain at extremely low frequency (near zero). Further, the presence of the IGA/IGA genotype would be expected to have no effect on wild pig population size since the differences are not statistically significant (see Figures A-22 and A-23 b and c).

Scenarios 23 and 24 (without carrying capacity), and Scenarios 31 and 32 (with carrying capacity) – an extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of South Carolina with a large wild pig population (50,000 individuals) with current and moderately higher prevalence of PRRSV (2 and 5%, respectively).

Scenarios without carrying capacity

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation, regardless of the prevalence of PRRSV (2% or 5%)—the IGA/IGA genotype stayed at an extremely low frequency (near zero) (Figure A-24a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size grew as expected over time (Figures A-24b, A-24c). Populations grew slower when the prevalence of PRRSV was greater (5% vs. 2%) but did not differ based on the presence of the IGA.

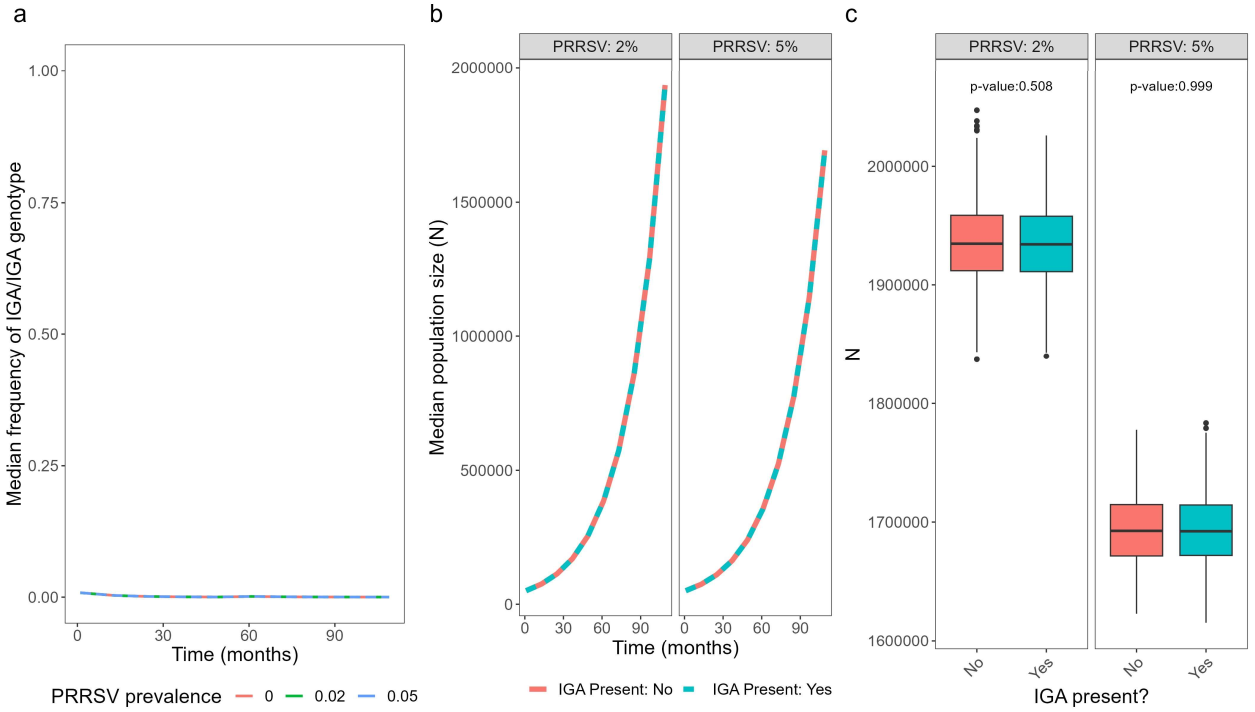

Figure A-24. Fate of IGA/IGA genotype and its effects on wild pig populations with no carrying capacity. Simulations consisted of an extremely large number of escapes (600 individuals) occurring at intermediate frequency (once every 5 years) in a part of South Carolina with a large wild pig population (50,000 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).

Scenarios with carrying capacity

The frequency of the IGA/IGA genotype behaved similarly to neutral genetic variation—the IGA/IGA genotype increased slightly with every escape event but remained at an extremely low frequency (near zero) over the entire 50-year period (Figure A-25a).

Wild population size was no different between scenarios with pigs containing the IGA and those that did not contain the IGA (control)—population size remained relatively stable over time (Figures A-25b, A-25c). Populations stabilized at a lower population size when the prevalence of PRRSV was greater (5% vs. 2%), but did not differ based on the presence of the IGA.

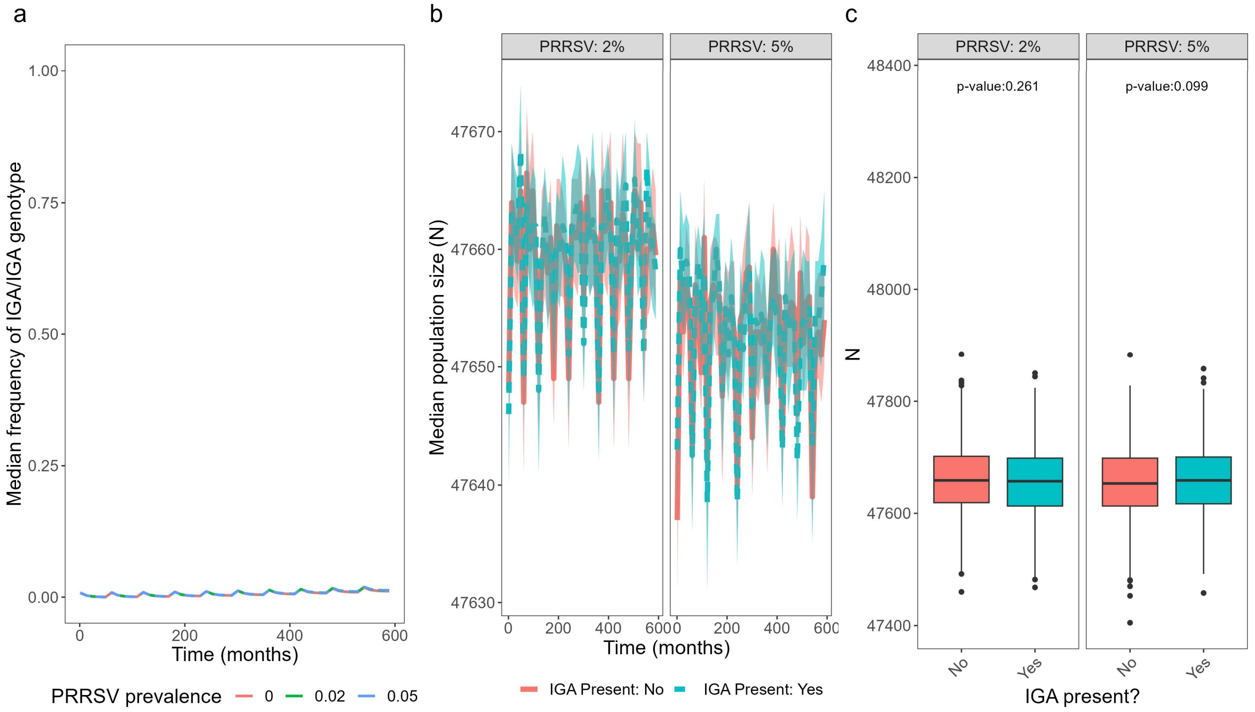

Figure A-25. **Fate of IGA/IGA genotype and its effects on wild pig populations with carrying capacity. Simulations consisted of an extremely large number of escapes (600 individuals) occurring at an intermediate frequency (once every 5 years) in a part of South Carolina with a large wild pig population (50,000 individuals) and moderate (2%) to high (5%) prevalence of PRRSV. Results include (a) frequency of the IGA/IGA genotype (prevalence = 0% is control), (b) wild pig population size over time, and (c) final population size (p-value based on unpaired t-test).**

In summary, if an extremely large number of pigs containing the IGA were to escape with an intermediate frequency (e.g., as a result of a natural disaster) in a location similar to South Carolina with a large wild pig population, the frequency of the IGA/IGA genotype would be expected to remain at an extremely low frequency (near zero). Further, the presence of the IGA/IGA genotype would be expected to have no effect on wild pig population size since the differences are not statistically significant (see Figures A-24 and A-25 b and c).

Conclusion

Simulation modeling was employed to evaluate the prediction that the escape of pigs with the IGA into wild populations would not result in any more environmental harm than pigs without the IGA as measured as an increase in wild pig population size. Simulation of a broad range of conditions, including those reflecting different wild pig populations (Texas and South Carolina), that represent reasonable conservative and reasonable worst-case scenarios indicate that the escape of the IGA into wild pig populations is not expected to significantly alter wild pig population size. This conclusion holds true even when the prevalence of PRRSV is almost double (5%) the maximum natural prevalence found currently in wild populations (3.4%).

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