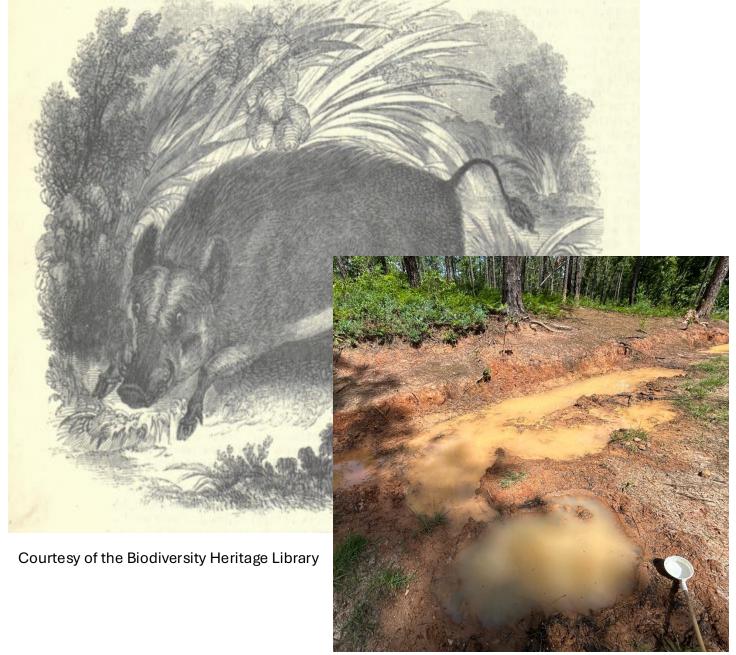


Wild Pigs (Sus scrofa)

- Invasive species
 - Population range and size increasing across the U.S.
 - Disruptive to the environment and human activities
- Ecosystem engineers
 - Rooting, spread invasive plants, alter runoff/watershed quality, wallowing
- Wallows
 - Filled with nutrients from pig excrement, and other organic materials from the disturbed earth
- Pathogen reservoirs
 - Japanese encephalitis virus (JEV)
 - Other zoonotic viruses



Pig wallow site, Savannah River Site

Wallows as Mosquito Habitat

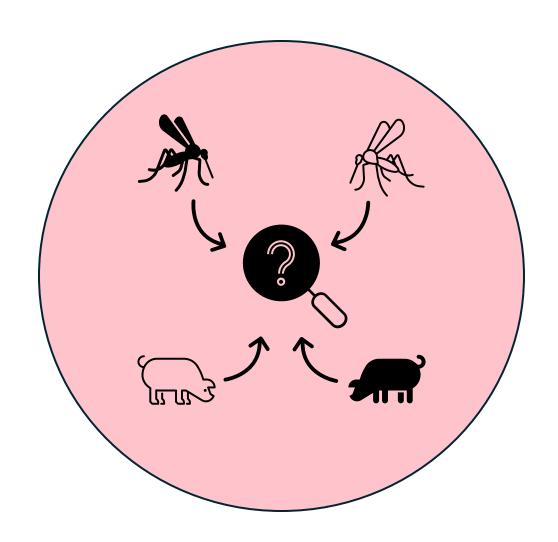
- Pig blood for female mosquitoes; pigs as pathogen reservoirs and amplifiers; wallows provide nutritious nurseries... a problematic bed-and-breakfast scenario
- This interaction can increase the spread of vectored pathogens



Mosquito larva in Savannah River Site pig wallow

Impacts of Pig and Mosquito Interactions

- Invasive wild pigs and *Culex* quinquefasciatus
 - Hawaiian honey creeper population decline
 - Avian malaria
- Gaps in understanding pig and vector mosquito related impacts on human and animal health



Mosquitoes of Interest

- Culex spp.
 - Japanese encephalitis virus
 - South-East Asia and West Pacific Region
 - Concern for emergence in North America
 - West Nile virus (WMV)
 - Avian malaria
- Anopheles spp.
 - Malaria
 - Recent locally transmitted cases in United States
 - Other arboviruses currently being explored

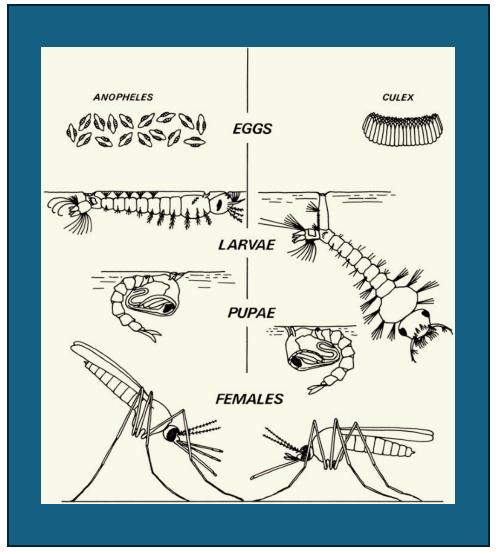


Fig. Image taken from Encyclopedic Reference of Parasitology. Springer, Berlin, Heidelberg.

Goa Determine mosquitoes interacting with both wild pigs and domestic pigs to assess exposure risk Measuring species and quantity of mosquitoes in wallows and pig barns Testing blood meals in mosquitoes for evidence of blood feeding and pig blood presence/absence of pathogen antibodies, primarily using WNV as a proxy for pathogens amplified in pigs

Methods

Sites:

- Pig barns with nearby wild pig activity, similar habitat types and varying insect exclusion measures
- Wild pig wallows near domestic pigs and our lab

Capture materials

- Standard CDC light mosquito traps (adults)
- Dippers (larvae and pupae)

ID and testing

- Identification of mosquitoes via standard references, barcoding
- Blood analysis
 - Wild and domestic pig blood in mosquito bloodmeals
 - Test for presence/absence of antibodies
 - ELISA
 - WNV



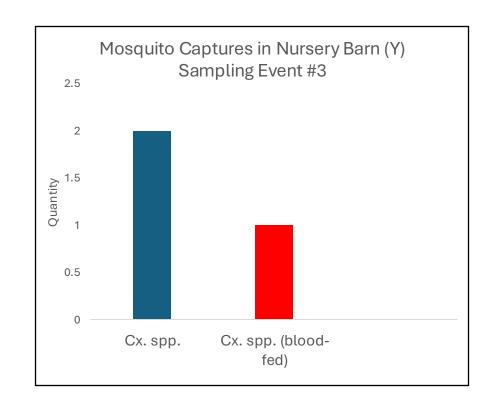






Preliminary Data

- Wallows
 - Both <u>Culex spp.</u> and <u>Anopheles spp.</u> present in wild pig wallows
- Barns
 - Mosquito species of interest, including blood-fed <u>Culex spp.</u>
 - Blood analysis is ongoing
 - Other mosquito species found inside and outside barns include <u>Anopheles spp.</u>
- Collections and data analysis are ongoing for this first season



Concern for Human Health

- Link between wild pigs, domestic pigs, and mosquitoes → carry over to humans
 - Wild pigs wallowing in proximity to human areas could increase vectoring mosquitoes near people
 - Mosquito attraction to pigs in general can lead to amplification and spread of some pathogens in domestic settings

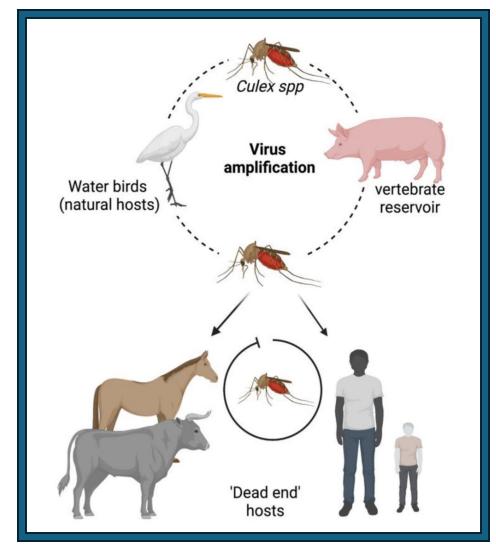
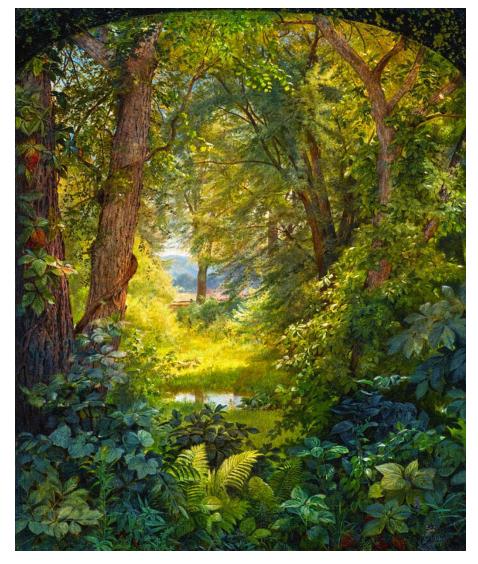


Fig. JEV cycle of infection and amplification from Srivastava KS et. al. Japanese Encephalitis Virus: An Update on the Potential Antivirals and Vaccines.

Concern for Ecosystem Health

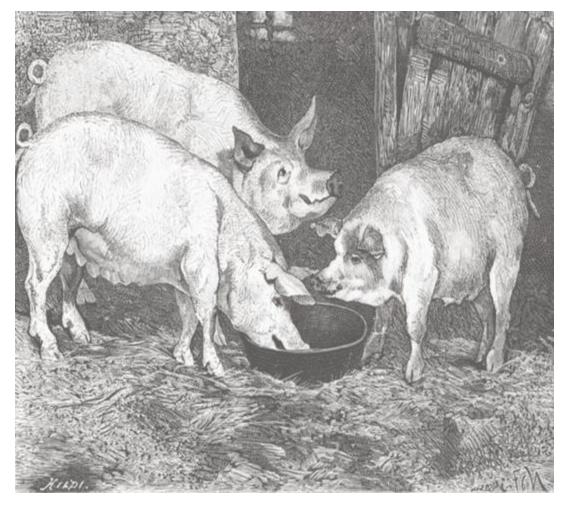
- Ecological synergism may have greater impacts than currently understood
- Potential co-expansion and interaction of wild pigs and vectors
 - Habitat destruction
 - Pathogen spread



Woodland Landscape Woodland Glade by William Trost Richards 1860

Concern for Agricultural Stability

- JEV limits reproductive success in boars and sows + medical complications/mortality in piglets
- Pathogen spread = substantial economic damage:
 - Should JEV arrive in the U.S., estimated economic loss = \$300-\$600 million per year



Drawing by Niederhausern.

One Health Implications

 All of these elements make for an excellent example of a one health study

 Understanding pig and mosquito interactions will help better inform risks to environmental, animal, and human health

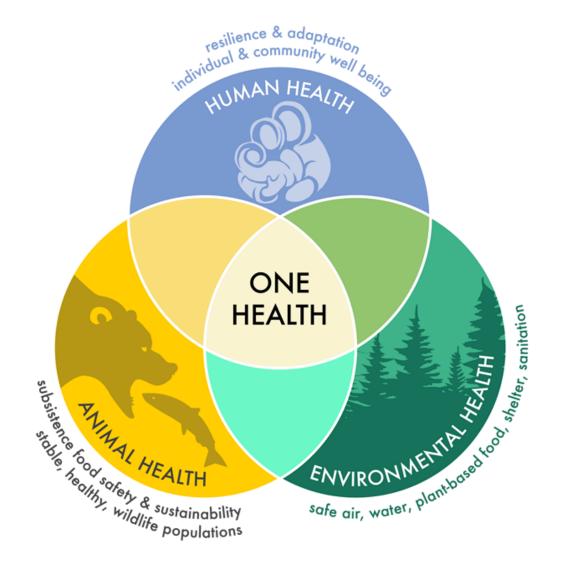


Fig. One Health graphic created by The University of Arizona

Future Directions

- Continue sampling in previous field sites
- Implement usage of mosquito aspirator backpack for more effective sampling around the pig wallows

 Diving deeper into viruses associated with mosquitoes utilizing pig wallows

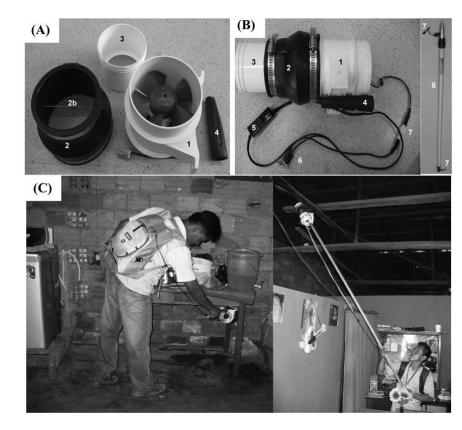


Fig. Cost-effective backpack aspirator by Vasquez-Prokopec et. al.

Acknowledgements:





 Russell Virden Utley – UGA Animal & Dairy Science





- Elizabeth Noblett Smithfield
- Arkadiusz Paczkowski Smithfield















Pathogens

- Culex
- JEV
- WNV

- Malaria
- EEV

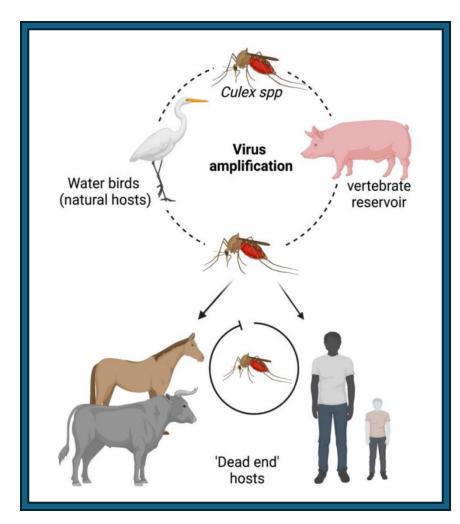


Fig 1. JEV cycle of infection and amplification from Srivastava KS, Jeswani V, Pal N, Bohra B, Vishwakarma V, Bapat AA, Patnaik YP, Khanna N, Shukla R. Japanese Encephalitis Virus: An Update on the Potential Antivirals and Vaccines. *Vaccines*. 2023; 11(4):742. https://doi.org/10.3390/vaccines11040742

Wild Pigs and Skeeters

- Wild pigs
 - Disruptive to human activities and environment
 - Disease reservoirs and amplifiers
 - Rooting and wallowing behavior disturbs earth
 - Depressions create nutrient-rich pools
- Mosquitoes utilize wallows
 - Breeding habitat
 - Blood meal from nearby pigs
 - Problematic "bed-and-breakfast" scenario



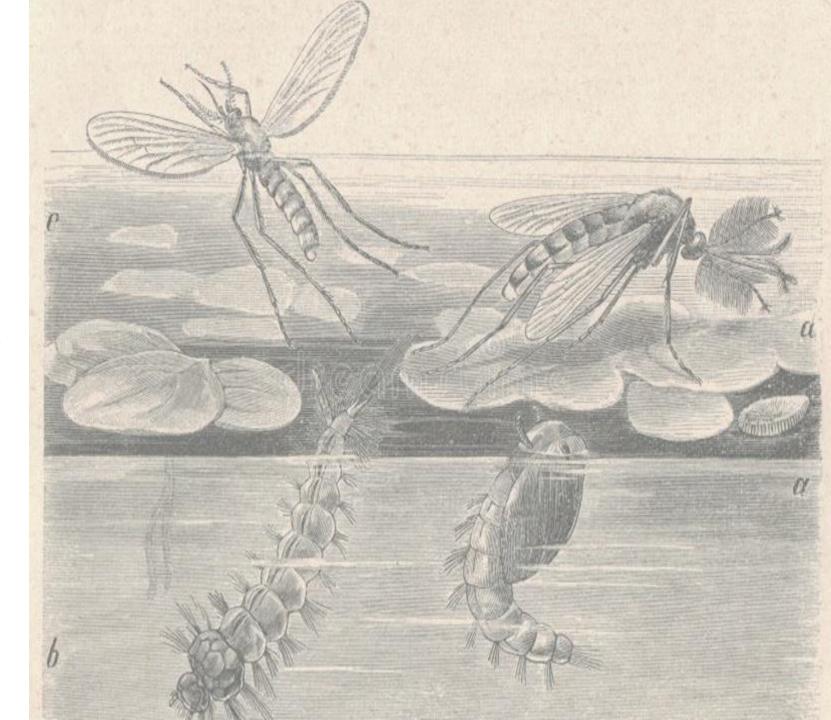
Wild Pigs (Sus scrofa)

- Invasive species
 - Population range and size increasing across the U.S.
 - Disruptive to the environment and human activities
- Ecosystem engineers
 - Rooting, spread invasive plants, alter runoff/watershed quality, wallowing
- Wallows
 - Filled with nutrients from pig excrement, and other organic materials from the disturbed earth
- Disease reservoirs
 - Japanese Encephalitis Virus (JEV)



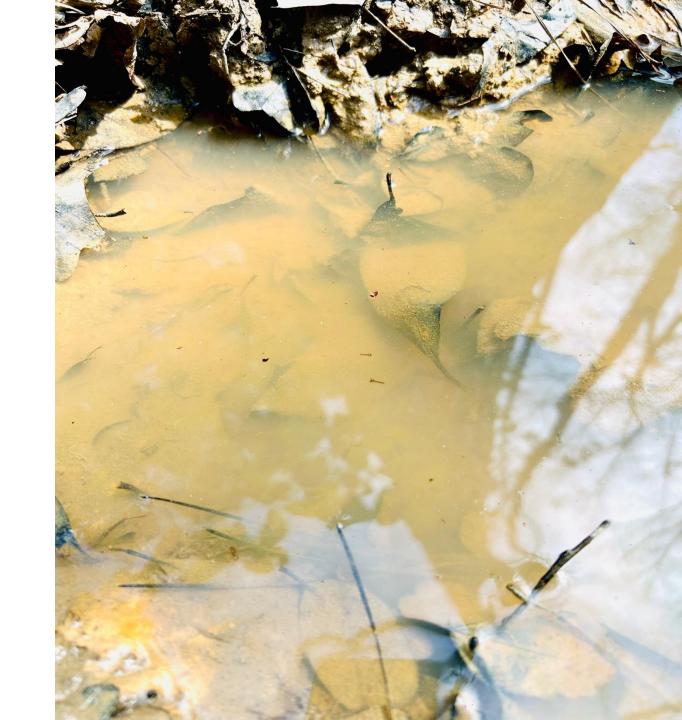
Mosquitoes (the Culicidae)

- Deadliest animal to humans
 - >700,000 human fatalities per year globally
 - Deadly to other animals
- Vectors of infectious agents
 - Transmit pathogens via blood feeding
 - (JEV)
 - Culex spp., of which many are invasive
 - Parasites that cause malaria
 - Anopheles spp.
 - Recent endemic malaria cases in U.S.
- Females require blood meals to produce eggs
- Mosquitoes require water to lay their eggs



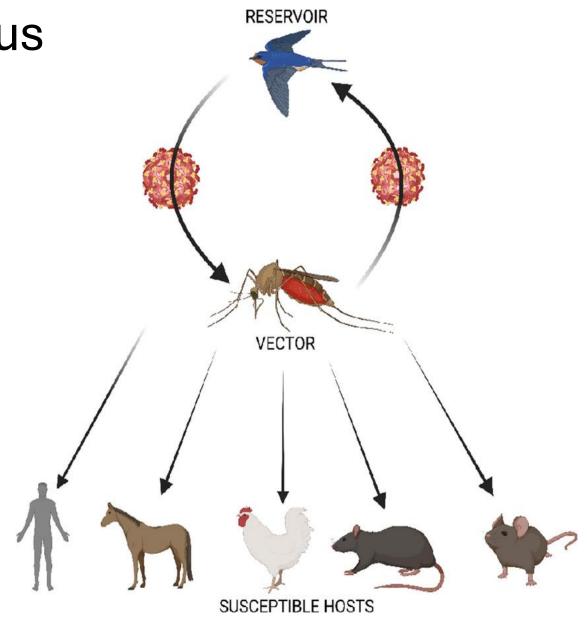
Wallows as Mosquito Habitat

- Pig blood provides mozzie females with sustenance; JEV amplifies in pigs; wallows provide nutritious nurseries: a bed-and-breakfast scenario
- This interaction can increase the spread of vectored pathogens
 - Creates breeding habitat and bloodmeal source for vectors
 - Invasive mosquitoes utilized invasive pig wallows in Hawaii, led to disease-related population decline



Japanese Encephalitis Virus

- An emergent pathogen
- Asia and the western Pacific region
- Exists in an enzootic transmission cycle
- Vectored by mosquitoes and amplified in pigs
 - JEV can cause fatalities in humans, as well as **limited reproductive success in boars and sows.**



enzootic transmission cycle

Methods

• Sites:

- Pig barns with nearby wild pig activity, similar habitat types and varying insect exclusion measures
- Wild pig wallows within the counties associated with pig barns, as well as SRS

Capture materials

- Standard mosquito traps (adults)
- Dippers (larvae and pupae)

ID and Testing

- Identification of mosquitoes via standard references, barcoding
- Blood analysis
 - Wild and domestic pig blood in mosquito bloodmeals
 - Test for presence/absence of antibodies
 - Using West Nile Virus as a proxy due to transmission cycle and antigenic similarities









Preliminary Data

- Mosquito species of interest, including blood-fed, found inside barns
 - Culex spp.
 - Blood analysis is ongoing
- Other mosquito species found inside and outside barns include *Anopheles spp.*, though these specimens are still being quantified and further identified.
- Collections and data analysis are ongoing for this season

Research Design & Aims:

Sites:

- 6 commercial sow farms and wean-to-finish farms in the southeastern U.S. as study sites. We will select sites with nearby wild pig activity, similar habitat types and varying insect exclusion measures in the southeastern U.S.
- <u>Aim 1:</u> I will establish the ecological relationship between invasive mosquitoes and invasive swine within the southeastern region of the U.S. / Establish whether wild pigs increase the habitat available to potential vectors of JEV by identifying the mosquito species that breed in wild pig wallows, in the U.S. southeast.
 - I will determine this by locating up to 5 wallows per representative habitat type and collect larval samples within every other week from March to October using a standard sampling technique with 350 mL dippers. My study sites will be chosen based on nearby wild swine presence and comparable habitat types. I will lab rear larvae until the 4th instar and then place them in ethanol morphologically identify them using taxonomic guides. I anticipate my results determining if mosquitoes utilize pig wallows, identification and abundance of those utilizing them, and the significance and efficacy of pig wallow sites as mosquito breeding habitats.
- Aim 2: Observe adult mosquito abundance to determine the contribution of mosquitoes developing in wallows to local mosquito diversity / Determine the extent to which mosquitoes access sow farms and wean-to-finish farms and what mosquito species are most commonly trying to enter facilities and get inside. We also will compare the effectiveness of existing insect exclusion methods for preventing mosquito access.
 - I will accomplish this by observing adult mosquitoes caught in CDC light traps, oviposition traps, and other glue and sugar traps nearby larval collection sites. To measure visitation pressure by mosquitos to swine production facilities, we will deploy disposable mesh traps coated in sticky-trap horse fly glue to windows, air vents, and above entranceways at swine farms. To measure successful mosquito access to swine farm facilities we will survey mosquito abundance and diversity within swine operation structures. As traps baited with synthetic host odor lures are unlikely to compete successfully with the attractiveness of live pigs, we will deploy a combination of CDC light traps, oviposition traps, and attractive sugar bait traps. Captured mosquitoes will be identified via morphological analysis and via PCR and compared to sequences in BLAST or VectorBase. The results of this analysis will aid in better projections of how wallows can change overall local mosquito communities.
- <u>Aim 3:</u> Elucidate potential negative contributions of mosquito breeding and feeding habits on mortality rates of native species, potential causes, and infer how wild pigs may intensify this effect / Assess how much exposure domestic swine herds currently experience to mosquito-borne pathogens present in wild pigs through blood-meal identification of mosquitoes collected in swine operations and testing samples of domestic and wild pig blood for evidence of pathogens.
 - I will assess the specific risk involved with hematophagous insects by blood-meal mosquito identification from the study sites and compare wild and domestic pig blood, with the help of swine researchers, for the presence of similar biological risks. These blood analyses will be successfully executed by well-established bloodmeal analysis and blood testing techniques. This will aid in determining specifics of how native wildlife would be affected by projected ecological function of hematophagous insects in conjunction with swine and animal-related disease mortality mechanisms.



Concern for Agricultural Stability

- JEV can cause fatalities in humans and other livestock, as well as limited reproductive success in boars and sows.
- Disease spread = substantial economic damage:
 - Ex. -> In 2002, West Nile Virus (mosquito-borne pathogen) emergence and transmission caused significant economic damage to equine industries
 - \$2.4 million in North Dakota, \$16.5 million in Texas, and \$2.75 million in Colorado and Nebraska.
- Should JEV arrive in the U.S., estimated economic loss = \$300-\$600 million per year

Concern for Agricultural Stability

- JEV leads to limited reproductive success in boars and sow, and medical complications or mortality in piglets.
- Pathogen spread = substantial economic damage:
 - Should JEV arrive in the U.S., estimated economic loss = \$300-\$600 million per year



Concern for Ecosystem Stability

- Invasive feral swine and mosquito species individually pose great threats to native ecology, but their unexplored ecological synergism may have greater impacts than currently understood and may result in a variety of disastrous consequences for local ecosystem stability.
- Ex. -> Culex quinquefasciatus mosquitoes breed in pig wallows and water-filled rooting depressions in Hawaii and transmit mosquitoborne pathogens, such as avian malaria, among many critically endangered Hawaiian birds. This has led to substantial avian population decline.
- Potential co-expansion and interaction of feral pigs and vectors may lead to local and foreign pathogen spread and habitat destruction which threatens local wildlife populations.

