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Review

Brief Review of Japanese Encephalitis Virus: Recommendations Related to North Carolina Swine Farms and Wider Implications for Swine Farming

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Abstract: Japanese encephalitis virus (JEV) is a mosquito-borne virus primarily found in Asia and Australia and is one of the few with an associated human/animal vaccine. Swine are amplifying hosts and wading birds are reservoirs of JEV, while horses and humans are incidental hosts. The primary vector is *Culex tritaeniorhynchus*, a generalist blood feeder not found in the United States (US); secondary vectors (e.g., *Cx. pipiens, Cx. quinquefasciatus, Aedes japonicus, Ae. vexans*) are widespread in the US (including North Carolina [NC]). The risk of JEV to NC was investigated because of widespread swine production, human populations, bird hosts, and possible mosquito vectors; however, recommendations can also apply to other swine producing states and regions. A brief review was conducted to identify transmission competent arthropod vectors, vertebrate hosts, and vector-host interactions for JEV. NC and other areas may be at risk for JEV emergence because of factors such as active international trade, volume of swine production, permissive climate, and widespread occurrence of potential vector species. Improved knowledge of the spatial distribution of swine farms, tracking movement of live swine, assessment of vector competence/capacity and blood feeding habits of potential JEV vectors, investigation of a JEV sentinel surveillance system, and assessment of efficacy for current biosecurity and control measures is needed to protect public and veterinary health.

Keywords: Japanese encephalitis; One Health; public health; risk assessment; veterinary health

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1. Introduction

Zoonotic diseases can be transmitted between animals and humans by direct contact, indirect contact, arthropod vectors, food, and/or water [1]. An estimated 60% of infectious diseases in humans can be transmitted from animals and 75% of new/emerging infectious diseases in humans originate from animals [1]. The global impact of zoonotic diseases on public health is significant and inevitable, hence, environmental/public/veterinary health professionals should focus attention on prevention of diseases through risk assessment and preparedness. A One Health approach including surveillance of zoonotic pathogens (e.g., viruses) and risk communication between public and veterinary health professionals, as well as agricultural operators is critical in protecting public and veterinary health by preventing and controlling zoonotic diseases, such as Japanese encephalitis [2]. Ideally, One Health integrates data from multiple affected groups and sources and this approach is improved with ongoing communication and collaboration between groups that establishes trust [2].

2. Global Distribution and Epidemiology of Japanese Encephalitis Virus

Japanese encephalitis virus (JEV; family *Flaviviridae*: genus *Flavivirus*) is a zoonotic mosquito-borne virus that emerged in Japan in the 1870s and was subsequently detected throughout eastern and southeastern Asia [3]. Currently, 24 countries in the Southeast Asian and Western Pacific regions are at risk for JEV transmission with a combined population of more than three billion people, including the US territory of Guam [4]. This virus is mostly prevalent in Asia (Russia, Japan, China, Southeast Asia, India) and Australia with a previously estimated 68,000 human JE global cases each year; however, more recent estimates are 100,000 cases per year [5–7]. The JEV genotypes I, II, and III are the most prevalent and distributed throughout Asia and Australia, accounting for 98% of detected strains from 1935–2009 [8]. Although JEV genotypes are generally isolated by region (i.e., genotype IV localized to eastern Indonesia and genotype V found primarily in Malaysia), some are emerging in new regions. Genotype V (usually restricted to Malaysia) has been found in Korea and China [8]. Cases of JE may be underreported; hence the annual number of human cases may be approximately 175,000, according to this report [9]. Typical human case fatality rates are 20–30% but can be as high as 67% and most fatalities occur in children and the elderly [10].

Swine are considered carriers and amplifying hosts and wading birds (Family Ardeidae) are primarily reservoirs for JEV but generally do not experience clinical symptoms (piglets can experience symptoms), although both can experience negative reproductive issues (e.g., abortion, stillbirth) that can lead to economic harm [11,12]. Swine can also serve as reservoirs, maintaining JEV and contributing to epidemics when mosquito vector populations are high during rainy and warm seasons [11]. Swine have a high JEV infection rate (98–100%) as shown in endemic regions and can maintain a viremia high enough to infect mosquitoes for four days post-infection but can clear the infection with loss of clinical symptoms over time [10,11]. JEV can be transmitted between and within populations of feral and domestic swine without vectors via mucous contact and aerosolized droplets [11,12]. Other studies have shown JEV can be transmitted via semen of wild boars, hence this may be another method of exposure between swine [13,14]. Competent mosquito vectors blood feeding on amplifying hosts can "bridge" JEV to humans and horses in subsequent blood meals. Humans and horses experience debilitating encephalitis from JEV infection and are dead end hosts, hence, mosquitoes cannot vector JEV from humans/horses to other animals. Human infection with JEV has the greatest impacts on children (<15 years) and the elderly [10,15]. Since humans are dead end hosts, human travelers are not a method for JEV being distributed to new areas. Cattle and other mammals (e.g., dogs, rodents) can also become infected with JEV; however, they do not serve as amplifying hosts [10]. Vectors of JEV include several *Culex* and *Aedes* spp. and the primary vector is *Cx. tritaeniorhynchus* Giles that are found in rice production areas with flooding irrigation in Asia and readily blood feed on swine and other animals including birds, cattle, and humans [4,10]. *Aedes japonicus* Theobald can also transmit JEV [16].

Although there is no treatment for the disease caused by JEV, antiviral drugs are in development [17] and supportive care to treat symptoms is available. Preventative human and animal vaccines are available in different endemic countries and are recommended. In NC, JE is listed as a vaccine-preventable disease (https://epi.dph.ncdhhs.gov/cd/diseases/vpd.html) (accessed on 5 October 2022) and vaccines (usually, the inactivated vaccine JE-VAX [licensed in Canada]-three doses needed for protection) are available for travelers who expect to visit JEV-endemic areas [18]. There is currently no licensed swine JEV vaccine in the US (https://www.cfsph.iastate.edu/pdf/shic-factsheet-japanese-encephalitis-virus) (accessed on 5 October 2022).

3. Risk of Japanese Encephalitis Virus: Imports and Exports

In areas at high risk of pathogen introduction and emergence, public health surveillance and risk assessments should occur, including investigating vector competence and blood feeding habits of local mosquito populations to prepare for the possibility of spillover from birds into swine and human populations. In China, JEV is endemic and established in swine and human populations, causing significant damage to public/veterinary health infrastructure and the economy [19]. In different areas of the US, the relationship between potential mosquito vectors and JEV emergence has not yet been fully evaluated. This should be done along with a cost–benefit economic evaluation for swine farms, wild swine, and preventative compared to reactive public health response efforts [20]. Pork exports from the US were valued at $7.7 billion in 2020 (66% of US exports went to China [$2.3 billion], Japan [$1.6 billion] and Mexico [$1.2 billion]) [21]. Each year, NC exports $650 million in pork and exports have increased (approximately 10-fold) in the past two decades (https://www.ncpork.org/exports/) (accessed on 5 October 2022). There is no evidence that JEV can be transmitted through processed pork products but higher demands on swine production could be a risk factor if JEV emerges in the US. Increased swine farm production may contribute to human JEV epidemics [11]. West Nile virus [WNV] was imported to Hawaii from mainland US [22] and, theoretically, JEV could also be introduced to the US via mosquitoes transported by humans or viremic vertebrate reservoir hosts. Migrating infectious birds could transport JEV from one region to another where local mosquito vectors could become infected while blood feeding on the birds [11]. The illegal importation of infectious birds is another route of entry into the US that could lead to JEV transmission to mosquitoes and other birds [20].

Changes in agricultural practices and animal reservoirs, climate change, international travel, the lack of mosquito-borne disease surveillance for arboviruses (particularly JEV) and other unknown factors have increased JEV risk [20,23]. International travel and trade increasing the movement of people and animals is one aspect of the One Health concept that interactions between people, animals, and the environment may enhance the spread of diseases [2].

In consideration of NC's current swine production levels (>2000 swine farms), some vector competent species of mosquitoes in NC, and the capacity and frequency of swine waste lagoon flooding adding *Culex* spp. oviposition sites, the risk of possible JEV transmission to NC deserves further evaluation. Current challenges in NC related to underfunded mosquito surveillance and control programs are cause for concern. This could lead to missed opportunities for detection and control of emerging zoonotic arboviruses. Hence, we have investigated the risk of JEV to NC because of widespread swine production, susceptible human populations, ample bird hosts, and possible mosquito vectors.

Most published studies show *Cx. tritaeniorhynchus* (primarily distributed in Asia) as the primary vector of JEV with swine as amplifying hosts and wading birds as primary reservoir hosts. *Culex pipiens* complex and *Ae. albopictus* (both widespread in the US) have also been identified as competent vectors of JEV [24]. Cattle, equines, reptiles, and humans can become infected with JEV, but are dead end hosts [24]. Hence, JEV can potentially be introduced into the US via importation of live infectious swine from regions with endemic JEV transmission. Currently, live swine can only be imported into the US from Canada and European Union member states with low risk of classical swine fever and an import permit and health certificate must be obtained from the USDA [25]. The US does not allow live swine to be imported from any other country due to the presence of foot and mouth disease, classical swine fever, African swine fever, bovine spongiform encephalopathy, or swine vesicular disease [25]. The US exports live swine largely to Mexico (receives 56% of US swine) [26].

Meat consumption in China remained relatively stagnant between 2016 and 2021 at approximately 75 million metric tons, while Chinese pork consumption fell from 57 million metric tons to 48 million metric tons during the same period [27]. Pork imports into China increased from around 3.25 million metric tons to >8 million metric tons in the same time frame [27]. This may indicate an insignificant change to dietary habits of the Chinese, but the demand for meat (pork especially) has increased. Due to pork price inflation, primarily caused by African swine fever virus, China must rely heavily on importing pork from the

US to mitigate food price inflation [28]. If JEV becomes an issue impacting and reducing US swine production, China would not be able to import as much pork from the US.

4. Mosquito-Host Interactions, Vector Competence, and Vectorial Capacity

Mosquito host feeding patterns, vector competence, and vectorial capacity impact enzootic and epizootic cycles. Others have shown 17 mosquito species capable of becoming infected with and transmitting JEV based on infected field-caught specimens coupled with laboratory vector competence assays: *Ae. albopictus* Skuse, *Ae. vexans* Meigen, *Ae. vigilax* Skuse, *Anopheles tessellatus* Theobald, *Armigeres (Ar.) subalbatus* Coquillet, *Cx. annulirostris* Skuse, *Cx. bitaeniorhynchus* Giles, *Cx. fuscocephala* Theobald, *Cx. gelidus* Theobald, *Cx. pipiens* Linnaeus, *Cx. p. pallens* Coquillet, *Cx. pseudovishnui* Colless, *Cx. quinquefasciatus* Say, *Cx. sitiens* Weidemann, *Cx. tarsalis* Coquillet, *Cx. tritaeniorhynchus* Giles, and *Cx. vishnui* Theobald [29,30]. Here, we focus on the primary vector (*Cx. tritaeniorhynchus*), emerging vector (*Ae. j. japonicus*), and vectors that may play a role if JEV emerged in NC (*Ae. albopictus*, *Ae. japonicus*, *Ae. vexans*, *Cx. pipiens*, *Cx. quinquefasciatus*). Infection, dissemination, and transmission rates for three strains of JEV were determined in *Cx. tritaeniorhynchus* and *Ae. j. japonicus* [16]. The same study showed that *Cx. tritaeniorhynchus* was a highly competent vector, and, while *Ae. j. japonicus* did not demonstrate a high level of vector competence, a small percentage became infected and transmitted JEV. European *Ae. albopictus* and *Cx. pipiens* were competent vectors for JEV and the extrinsic incubation period was shorter in *Ae. albopictus* compared to *Cx. pipiens* [24]. Another study showed one population of *Cx. quinquefasciatus* collected in Georgia (North America) could become infected with and transmit JEV at a low rate [31]. A follow up study on *Cx. quinquefasciatus* from the same region of Georgia showed this population could transmit either genotype I or III JEV strains and were better vectors of some strains (i.e., genotype Ib and genotype III) than others [32]. There is limited published information about vector competence of *Ae. vexans* for JEV. No known vector competence studies have been conducted on field populations of NC mosquitoes. Vector capacity should also be considered for JEV, i.e., mosquito-host contact, abundance, survival rates related to environmental conditions and/or impacts of mosquito control [30].

Host feeding preference is also important when evaluating the risk of enzootic and/or epizootic virus transmission in animals and epidemic transmission in humans. Information follows for NC mosquitoes capable of transmitting JEV (*Ae. albopictus*, *Ae. japonicus*, *Ae. vexans*, *Cx. pipiens*, *Cx. quinquefasciatus*). *Ae. albopictus* are opportunistic blood feeders, blood feeding on a variety of avian and mammalian species (including swine and birds). A multi-year study in Pennsylvania showed *Ae. japonicus* preferentially blood fed on mammals (e.g., deer, cat, dog, human, rabbit, cow, horse) [33]. A New Mexico study found *Ae. vexans* also fed primarily on mammals (e.g., cows, horses, rabbits) but also fed on birds (e.g., goose, duck, emu, robin) [34]. The same study found that *Cx. quinquefasciatus* blood fed primarily on birds (e.g., hawk, goose, duck, finch, robin, sparrow, pigeon, etc.), but also fed on mammals (e.g., cow, coyote, dog, goat, donkey, horse, human, skunk, mouse, sheep, rabbit, panda, arctic fox, water buffalo, camel, rhinoceros, hippopotamus, red river hog) and host preference for this species is strongly influenced by seasonal variability and host availability. Due to overlapping blood feeding patterns observed and vector competence, several mosquito species could serve as bridge vectors for zoonotic diseases in the US and elsewhere [35]. It is currently unknown whether NC mosquitoes can vector JEV and this should be assessed.

It is important to understand potential interactions between feral and confined swine and mosquitoes (part of vector capacity). For instance, to what extent does swine housing minimize or prevent mosquito exposure and blood feeding and how would those interactions differ in feral swine? Do swine housing conditions vary between different types and locations of farms and how might differences impact risk assessments? This is currently unknown for US/NC and there are limited studies in other regions. JEV can be found in rural, suburban, and urban areas and different mosquito species may play a role as vectors in these different environments [12]. Increased urbanization may increase instances of swine-human contact, hence we must continue to improve our risk assessments to protect health [12]. A study of Cambodian farmers showed only 15% of respondents protected their pigs from mosquitoes [36]. In Nepal, lack of financial support and infrastructure limited swine farmers' ability to house pigs indoors to potentially limit mosquito bites [37].

5. Swine Farms in North Carolina

North Carolina has a rich agricultural history ranging from cotton, tobacco, and sweet potatoes, to turkey, cattle, and swine. As the State's agricultural landscape has changed over decades, swine farms have undergone dramatic transformation. From the mid-1900s to 1990s, small local swine farms have been replaced with large industrial swine farms [38]. This change resulted in a decrease in quantity of farms while increasing swine production [39]. During the 1990s, eastern NC experienced a resurgence of swine farms as the tobacco industry declined [40]. Swine are distributed throughout thousands of small farms across the southeastern and midwestern US [40]. However, during the 1990s, the total number of swine farms across eastern NC declined ca. 42% [40]. Despite the decline of farms, swine production increased during the same period [41]. This was likely due, in part, to the transition period from small farms to large scale industrialization of swine production in confined animal feeding operations [CAFOs] [41]. The USDA estimates there are currently (in 2022) approximately 2100 swine farms in NC (8,000,000 swine) and the NC Pork Council estimates more than 2100 swine farms producing 9,000,000 swine (https://www.ncpork.org/exports/) (https://usda.library.cornell.edu/concern/publications/rj430453j?locale=en) (accessed on 5 October 2022) located predominantly in the southeastern region of the state (Figure 1). The USDA ranks NC third in US swine production (the NC Pork Council ranks NC second in swine production), with Iowa (23,000,000 swine) ranking first and Minnesota (8,600,000 swine) ranking second (https://www.ncpork.org/exports/) (https://usda.library.cornell.edu/concern/publications/rj430453j?locale=en) (accessed on 5 October 2022). No information is publicly available on farm scale, husbandry practices, and prevalence and proximity of wild swine populations that would inform risk assessments for JEV and this is needed.

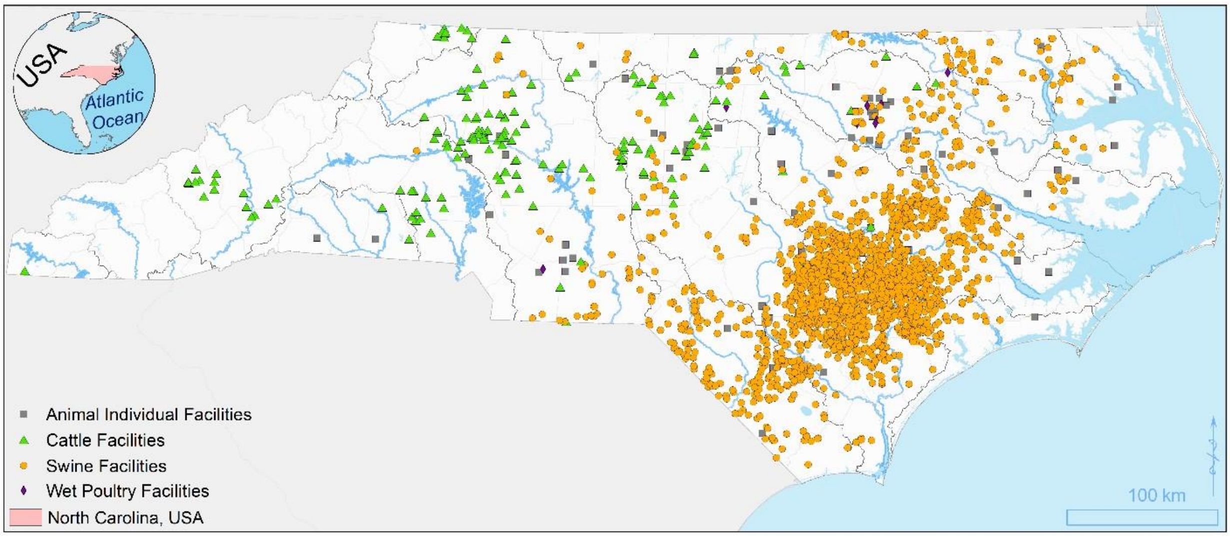

Figure 1. Map of permitted swine (yellow dots: state certificate of coverage [COC]; light purple: National Pollutant Discharge Elimination System [NPDES] COC) facilities in NC (Permit data obtained from NC Division of Environmental Quality 2020). Note that cattle and poultry farms are also indicated with different colored dots (see legend).

Waste lagoons that detain wastewater from swine farms have increased in quantity and size due to the increase in size of CAFOs [42]. Furthermore, commercial-scale swine farms require large tracts of land with gentle topographies typical of low-lying coastal regions. Thus, in NC and other hurricane-prone areas, hurricanes, heavy rainfall, and/or poor maintenance can result in flooding/overflow issues in swine lagoons [41] and this has resulted in lawsuits from local homeowners due to air and water quality concerns [42]. As a result of nuisance odor complaints, some farms may begin large scale adoption of covered lagoons, that if designed or maintained poorly, can facilitate ponding of rainwater on lagoon covers, thereby creating oviposition sites. Flooded overflow areas of lagoons may provide oviposition sources for mosquito species that prefer to oviposit in standing water with high organic material (e.g., *Culex* spp.) [43]. The possibility of CAFOs providing oviposition sites for potential swine vectors should be assessed.

The CAFOs dispose of waste in varying forms, depending on animal type. Horse farms produce more solid waste than swine farms due to bedding, concentrated feces, and feed lots [44]. Swine farms use lagoons to store liquid waste and periodically extract liquid wastewater to spray waste on surrounding fields using irrigation systems for soil fertility [45]. Sunlight reduces pathogens that may be present in the waste. However, over spraying and inconsistent light exposure may allow pathogens to enter groundwater and/or become aerosolized [45]. This could be another potential avenue of JEV exposure as it can be transmitted via aerosol and contact (without arthropod vectors) shown in mice [46] and swine [47]. Oronasal shedding [47] is low level and transient so is only a risk for swine-swine transmission in confined spaces. If considering wider risks to human and animal populations through effluent which is currently unknown, a detailed risk assessment would be required considering the quantity of virus released, dilution factor within the effluent, virus survival, and likely exposure.

Swine farm waste is of particular concern under severe weather conditions. In 1995, rainfall-related flooding resulted in an Onslow County (eastern NC) swine farm waste lagoon breach and eventual collapse of a retention wall, causing > 95,000 metric tons of feces/urine to overflow into fields and the New River [48]. In 1999, eastern NC experienced Hurricane Floyd, resulting in $\geq$ 46 animal waste lagoons flooding and contaminating surrounding areas [39]. As CAFOs generally are large (thousands of animals at one location), waste lagoons cover multiple acres [49]. As of 6 November 2019, there were 2376 permitted animal facilities that practice animal feeding operations in NC [50]. The majority of CAFOs produce swine and are in eastern NC between Rocky Mount and New Bern/Pamlico Sound [50]. The CAFOs are more concentrated in coastal areas with lower geographic elevation, hence are prone to flooding. Impacts from flooding can occur for weeks, creating ideal oviposition sources for *Culex* mosquitoes and this should be considered in risk assessments.

6. Tracking Swine Farms and Risk Communication with Farmers and Veterinary/Public Health Professionals

The Food and Agricultural Association (FAA) of the United Nations tracks global swine distribution [51], hence this could potentially be used as a basis for a JEV tracking and/or vaccine distribution system, if needed. A database promoting information sharing (e.g., symptoms of JEV infection in humans and animals) to farmers and public/veterinary health professionals currently does not exist and could be promoted. Furthermore, a publicly available database showing biosecurity measures in place for farms in different US states and/or regions is currently not available.

In NC, a pilot program offered through NC Department of Agriculture and Consumer Services (with collaboration from State Veterinarian) is underway and loans participants traps for feral swine (https://www.ncferalswine.org/five-county-trap-loan-project) (accessed on 5 October 2022). The same program works with participants to get the trapped swine tested for different diseases. There is also a mechanism in place where community members can report feral swine and the damage they have caused (https://www.ncferalswine.org/) (accessed on 5 October 2022). Although there is an ongoing campaign by the US Department of Agriculture highlighting the dangers of African Swine Fever virus (not a vector-borne disease) (https://www.aphis.usda.gov/aphis/resources/pests-diseases/asf/asf) (accessed on 5 October 2022), there is no such campaign for JEV. Swine vaccination occurs in endemic Asian countries, but is not widespread, likely due to cost and lack of JEV knowledge. Swine vaccination can be beneficial to prevent reproductive issues such as abortion or stillbirth that could cause economic harm. There is not a licensed JEV vaccine for US swine (https://www.cfsph.iastate.edu/pdf/shic-factsheet-japanese-encephalitis-virus) (accessed on 5 October 2022). A Cambodian study showed limited knowledge of JEV transmission in swine farmers, i.e., did not know mosquitoes transmit JEV or that pigs could become infected [36]. In Nepal, knowledge of JEV and preventative practices varied widely among swine farmers related to factors such as literacy, gender, and culture [37]. Additional studies would need to be conducted to compare the swine farming practices between these underdeveloped countries and the US. No such published study exists for NC or other US swine farmers and would be useful for risk assessments.

7. Discussion

We have demonstrated that in NC there is potential risk of JEV because of factors such as density of swine in close proximity in CAFOs, unknown frequency of potential transmission within wild swine and bird populations, permissive climate, potential for importation of infectious mosquitoes or vertebrate hosts, and widespread potential vector species. However, NC is not necessarily unique (except for its position in a hurricane-prone region that results in lagoon flooding) and JEV risk could be broadened to apply to any other US state or region with higher (e.g., Iowa) or similar (e.g., Minnesota) levels of swine production and permissive factors such as competent vectors and that should be explored in a future study. The current study explores the possibility of JEV transmission in NC and highlights several knowledge gaps. To date, JEV has not been identified in the US. The primary JEV vector (*Cx. tritaeniorhynchus*) is native to Asia and the South Pacific islands and has also been found in Africa and southeastern Europe [52] but is not found in the US. However, secondary vector species (i.e., *Ae. albopictus* [invasive species from Asia], *Ae. japonicus* [invasive species from Asia], *Ae. vexans, Cx. pipiens, Cx. quinquefasciatus*) are prevalent in NC and other areas of the US [24,53]. A previous risk assessment of JEV introduction into the US was rated as negligible; however, changes in environmental conditions and other factors influencing pathways of introduction may change this rating and should be re-evaluated periodically for the US [20], including NC. Others have developed machine learning systems to assess risk for another swine disease (porcine reproductive and respiratory syndrome virus—not vector-borne) [54]. The same study categorized farm risk based on biosecurity and demographics with increased risk associated with factors such as sharing trailers used to haul animals, high employee turnover, and high numbers of swine in the local area. A similar approach could be taken for JEV risk assessment in NC and other regions based on transmission dynamics (i.e., risk models informed by factors such as mosquito vectors, employee turnover, swine [domestic and feral] density in the area, insecticide treatment measures or repellent use by farm owners and employees, and other factors). In NC, there are regulations in place so that live swine transported on public roads not having identification would result in a civil penalty through NC Agriculture and Consumer Services (https://www.ncagr.gov/vet/Livestock/SwineIDandImportation.htm) (accessed on 5 October 2022). Transported swine not having identification (i.e., ear tag) are considered feral and are regulated by the Wildlife Resources Commission. Biosecurity programs can be broadly focused and include prevention measures for a variety of swine diseases [55], hence JEV should be included on the list of potential pathogens for swine in NC. The diagnostic capability of public and veterinary health professionals for JEV in NC and elsewhere in the US is currently unknown and should be considered in risk assessments. Asymptomatic cases, short duration of viremia, and infectivity of JEV causing biosafety level three precautions to be taken could be an impediment to testing and diagnosis [18]. The Centers for Disease Control and Prevention's publicly available surveillance system for mosquito-borne diseases currently does not list JEV (currently listed mosquito-borne pathogens are WNV, St Louis encephalitis virus [SLEV], Eastern equine encephalitis virus [EEEV] Jamestown Canyon virus [JCV], La Crosse virus [LACV], dengue virus [DENV], chikungunya virus [CHIKV], Zika virus [ZIKV]), hence this is not being routinely tested for and tracked via mosquito pools, human, or veterinary testing (https://wwwn.cdc.gov/arbonet/maps/ADB_Diseases_Map/index.html) (accessed on 5 October 2022). In NC, there are primarily imported human cases of disease caused by DENV, CHIKV, and ZIKV, ongoing local transmission of WNV, EEEV, and LACV, and historical cases of SLEV and JCV. Since a publicly available database of biosecurity measures on swine farms does not currently exist in NC, the US, or elsewhere, this could be developed at least at the state level to facilitate risk communication and help prevent pathogen spread. This database could include biosecurity levels of farms in the state and information related to mosquito prevention methods of farmers (e.g., insecticide application, repellent use).

Recommended components of JEV risk assessment in NC that could be tailored for other regions that future studies could investigate include: (1) maintaining up-to-date maps within a geographic information system including locations of swine farms, (2) tracking system for imports of live swine from JEV-endemic regions (including routine serology testing), (3) JEV vector competence and vector capacity assessments for commonly found local mosquito populations (e.g., *Cx. pipiens*, *Cx. quinquefasciatus*, *Ae. albopictus*, *Ae. japonicus*, *Ae. vexans*), (4) host feeding preference field studies near swine farms to determine the extent to which these mosquito species blood feed on swine (confined and feral), (5) analysis of the potential interaction between confined and wild swine and mosquitoes; are swine kept in buildings not exposed to mosquito feeding or not and how does this vary between different farms, tracking occurrence of wild swine, and (6) information sharing to farmers and public/veterinary health professionals detailing swine and human symptoms of JEV infection; logistical plan for acquiring/administering the JEV vaccine to swine and possibly humans living in swine producing areas.

8. Conclusions

It is possible a JEV emergence in NC could cause significant economic harm and health issues for humans and native fauna in the US since the population is naïve. However, previous exposure to related flaviviruses such as WNV (and thereby antibody presence) in these areas may provide some protection, although this is currently unknown [10]. Swine production is prevalent in eastern NC and other US states and preparation for the possibility of JEV emergence and other pathogens that may be introduced should be investigated further. These biosecurity measures will protect swine health, farm productivity, and public health [55]. This can be done by conducting routine mosquito and JEV surveillance, providing education on availability of human vaccines and JEV symptoms, and opening communication between swine farmers, public health professionals, and veterinarians about the possibility of JEV emergence. In any case, collaboration should be encouraged between health professionals, academic researchers, and farmers engaged in animal agriculture, following the One Health approach to solving health issues related to multiple sectors [56]. Partnerships between affected sectors will improve human health, food security, as well as animal health and environmental health [56]. Health professionals should stay on alert to prevent JEV transmission and/or geographic spread.

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