SHIC report

HPAI risks to swine and knowledge gaps for prevention and preparedness of H5N1 infections in the swine industry

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Introduction

The recent emergence of high pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b in dairy and the ongoing outbreaks of HPAI in commercial poultry seriously threaten the U.S. swine industry. Due to the role that pigs play in the overall ecology of influenza infections, the potential risk of H5 infections in people, and the epidemiological links between swine, dairy, and poultry, it is of the utmost importance to fully understand the risks to pigs and from pigs to other species, including humans. Of equal importance is the development of science-based strategies needed to prevent the introduction of H5N1 into pigs and contain it should incursions occur.

HPAI H5N1 first appeared in 1996 in Guangdong, China (Chen et al., 2004; Claas et al., 1998; Subbarao et al., 1998). Since then, it spread globally causing large outbreaks in multiple avian and mammalian species, and transmitting silently in others facilitating its ongoing spread, evolution, and reassortment (Jhung et al., 2015; Spackman et al., 2016). In 2020, HPAI clade 2.3.3.4b H5N1 emerged, became panzootic and in 2021 was introduced into North America causing infections in wild birds. From there it spread to commercial poultry, wild terrestrial and marine mammals, and farmed animals. Since 2020, more than 48 mammalian species have been reported infected (CDC, 2024b; Graziosi et al., 2024; Plaza et al., 2024; Webby & Uyeki, 2024).

Since the start of the outbreak in February 8, 2022, 103.47 million birds have been affected as of October 17, 2024 causing significant losses to the poultry industry (*Confirmations of Highly Pathogenic Avian Influenza in Commercial and Backyard Flocks | Animal and Plant Health Inspection Service*, n.d.). In March 2024, the first case of H5N1 clade 2.3.4.4b was confirmed in dairy cows in Texas (Burrough et al., 2024). As of October 17, 320 cases of H5N1 clade 2.3.4.4b have been reported in 14 states causing significant economic losses to the dairy industry and representing an ongoing threat to other animal species (*HPAI Confirmed Cases in Livestock | Animal and Plant Health Inspection Service*, n.d.). The initial introduction of the H5N1 2.3.4.4b clade genotype B3.13 into dairy cattle is thought to have occurred as a result of a single transmission event that took place in late 2023 likely from a wild avian species (Nguyen et al., 2024). At first, reports of reduced feed intake and rumination, an abrupt drop in milk production, and changes in milk quality (e.g., thicken, creamy) were reported in multiparous cows during the middle to late lactation (Burrough et al., 2024). Clinical signs of fever with minor respiratory

signs (e.g., nasal secretions, coughing, etc.) with minimal death were reported occasionally. On affected dairy herds, fatal systemic influenza infections were also reported in domestic cats that had consumed unpasteurized raw colostrum and milk from infected cows. After that, local and widespread on-ward transmission, facilitated by the interstate movement of cattle, has been documented as the major drivers of disease spread (Nguyen et al., 2024). Transmission of H5N1 from cows to other mammals, such as mice, fox, and raccoons, has also been documented (Caserta et al., 2024; Peacock et al., 2024). Cases in humans have also been reported, mostly associated with occupational exposure during flock depopulation events (poultry) or milking activities in parlors, and one case had an unknown source of exposure. Symptoms in infected humans include conjunctivitis, fatigue, and mild respiratory symptoms, from which they recover (CDC, 2024d).

The widespread distribution of H5N1 infections in wild birds, poultry, multiple mammalian species, and now in dairy cattle undoubtedly has increased the risk of exposure for pigs and transmission to people. This summary discusses some knowledge gaps regarding HPAI H5N1 clade 2.3.4.4b relevant to pigs. Ultimately, this summary attempts to help the swine industry to prepare for and prevent H5N1 influenza infections in pigs.

Experimental infections of H5N1 in dairy cattle and pigs

A key aspect of disease preparedness is to recognize how the disease presents in infected animals. Experimental infections in calves, heifers, and lactating cows confirmed the virus can infect the animals through aerosol, oral, and intramammary inoculation routes (Baker et al., 2024; Halwe et al., 2024). In general, infection of heifers and calves is limited with mild to moderate respiratory disease with limited clinical signs, lesions and on-ward transmission. In contrast, clinical disease in lactating cows is significant resulting in high fever, decreased rumen motility, changes in milk appearance, production losses, and some nasal discharge (Baker et al., 2024; Halwe et al., 2024). High virus titers (10^8 TCID50/ml) can be found in milk associated with severe acute mammary gland infection and necrotizing mastitis; however no evidence of systemic infection outside the mammary gland or respiratory tract and associated lymph nodes was found (Halwe et al., 2024). Investigations are necessary to ascertain whether pigs can become infected through the mammary glands and whether this infection can spread systemically. Infections in lactating animals including sows, are needed to assess H5N1 viral shedding in sow's milk and its potential risk for infection to suckling pigs.

To date, experimental infections with H5N1 2.3.4.4b B3.13 genotype, i.e., the dairy genotype infecting cattle in the U.S., have not been conducted in pigs. However, few studies using other H5N1 viruses indicate the ability of the pigs to become infected with H5N1 viruses resulting in variable clinical presentations, lesions, and transmission risks depending on the H5N1 virus genotype and animal species origin of the virus (Lipatov et al., 2008; Graaf et al., 2023; Kwon et al., 2024; . Arruda et al., 2024). Of special concern are those infections where pigs showed

neurologic signs despite what otherwise would be mild clinical signs (Arruda – personal communication) and where virus can replicate and/or be detected in the brain, lower respiratory tract, feces, and pancreas indicating systemic infection. There is evidence for increased replication and transmission of H5N1 from mammalian, and potentially selected avian isolates, to pigs which stresses the importance of recognizing a broad range of clinical signs associated with H5N1 infections. Studies are necessary to ascertain whether genotype B3.13 is capable of causing clinical signs in pigs, replicate, shed, and if so how, and for how long, cause lesions and, consequently, transmit to other animals. A key question is whether infection with this genotype will be restricted to the respiratory tract similar to most swine influenza isolates, or whether it will have the ability to be systemic and cause widespread infection. Furthermore, understanding the source of virus introduction into pigs will be necessary particularly whether similar to dairy, it is the result of a single introduction, or multiple introductions. This is important because the introduction and establishment of distinct H5N1 genotypes in pigs could result in a complex epidemiological picture that could favor the emergence of novel reassortant viruses with different clinical presentations and degrees of virulence. However, it is unknown how the introduction of H5N1 infections in populations infected with endemic swine influenza viruses, or other swine viruses, would manifest clinically. Answers to these questions are crucial to inform and design effective surveillance, prevention, and control H5N1 programs in pigs. Of concern is that most influenza surveillance efforts are voluntary and sample submissions could be halted should the risks of detecting H5N1 in pigs not be managed appropriately.

Field investigations of H5N1 2.3.4.4b in dairy cattle, pigs and peri-domestic animals

In addition to experimental infections, the importance of field investigations cannot be overstated. Reports of clade H5N1 2.3.4.4b in dairy herds (Caserta et al., 2024) shortly after cases were confirmed supported observations of cow-to-cow transmission associated with the introduction of asymptomatic / subclinically infected cows from infected herds within and between states. Similar clinical signs and production impacts to those observed at the beginning of the outbreak were reported. Interestingly, the detection of viral RNA in nasal swabs and urine took place more often in non-clinical, what veterinarians and producers considered "healthy" animals, than clinical "sick" animals. Nasal shedding was estimated to last about 7 days and shedding in milk lasted for about 31 days with infectious virus recovered in milk for up to 16 days approximately. Viral titers in milk were high - between 10^4.4 and 10^8.8 TCID50/ml and seroconversion was widely distributed. Multi-directional interspecies transmission between dairy cows, birds, domestic cats, and a raccoon was also documented on infected herds which raises concerns about the potential for some of the species to become reservoirs thereafter. Should H5N1 be detected in pigs, investigations to understand the dynamics of within-herd transmission in pigs, including herds with different disease statuses would be needed. This information should support diagnostic strategies to enable producers to implement control measures such as segregation, culling, enhanced biosecurity, or increased monitoring.

Field observations have documented H5N1 infections in pigs in various parts of the world. These infections were caused by a range of distinct H5N1 genotypes and resulted in variable degrees of seroconversion and clinical disease and affected commercial and back-yard pigs. (Choi et al., 2005; Mahardika, 2018; Meseko et al., 2018;Rosone et al., 2023;. Hervé et al., 2021). The possibility of pigs being naturally infected with H5N1 2.3.4.4b genotype B3.13 should be considered a very likely event in particular in areas where feral pigs and outdoor pigs may come in contact with wild animals or extensively raised domestic animals. Thus, enhanced surveillance efforts should be in place in backyard pigs and domestic pigs with outdoor access, to identify and contain potential infections in pigs.

Furthermore, the duration of influenza infections in pigs may vary based on population size, immunity, dynamics, and presence of co-circulating viral strains (Y. Li & Robertson, 2021; Van Reeth & Vincent, 2019). Infections may die out quickly in small-sized populations (Janke, 2013), or become endemic in large populations, where the herds become a continuous source of influenza viruses (Diaz et al., 2015, 2017). Similarly, pigs may be infected with the same or distinct virus strains which may affect the duration of shedding, risk of co-infections, and risk of having novel reassortants (Allerson et al., 2013; Nirmala et al., 2021). It will be important to determine how H5N1 infections in pigs behave based on herd size and immunity, particularly. This information will be crucial to evaluate whether control strategies such as prolonged gilt isolation, herd closure, or vaccination can be effective in controlling and eliminating H5N1 viruses in pigs.

Similarly, determining the prevalence of H5N1 genotype B3.13 in wildlife and peri-domestic species should remain an area for ongoing surveillance. As infections continue to spread to more regions, new animals, in particular peri-domestic species near poultry and dairy farms may become infected (*HPAI Confirmed Cases in Livestock | Animal and Plant Health Inspection Service*, n.d.). Increasing prevalence rates in peri-domestic animals is particularly worrisome for pigs because these peri-domestic species have the potential to interface with both feral and domestic pig populations. Seasonal considerations in terms of species habitat and access to food may drive migration patterns of some peri-domestic species which may facilitate seeking indoor spaces during cold weather seasons. Furthermore, regional considerations in terms of feral pig populations, type of pig production (indoor vs. outdoor), and participation in agricultural fairs and exhibition shows should also be evaluated to fully assess the risk of H5N1 incursions into pigs. Endemic swine influenzas are effective at spreading through the "show pig circuit" and incursions of H5N1 into this population could be significant for the interstate transmission of H5N1 in pigs and other species (Brophy et al., 2024; McBride et al., 2021; Szablewski et al., 2024). A full understanding and quantification of transmission risks through these alternate channels and from peri-domestic species to pigs is needed.

Transmission through indirect routes

In addition to direct transmission through the introduction of infected animals, indirect transmission through fomites, such as vehicles, equipment, and people, has been a suspected risk factor in poultry and dairy farms (Caserta et al., 2024; Hu et al., 2024; Nguyen et al., 2024; Singh et al., 2024). It is unclear what sort of epidemiological links exist between dairy, poultry, and pigs but proactively identifying the pathways of infection and assessing the risk of transmission should be performed. The results can be used to design targeted risk mitigation measures to prevent H5 virus introduction in pigs. More specifically, sharing of labor, equipment, and services among farms - regardless of species - and including backyard and non-commercial production systems, should be investigated to understand disease transmission risks and design effective biosecurity protocols.

There are numerous biosecurity protocols that are considered standard for the U.S. swine industry. For example, protocols for supply entry such as the use of UV boxes are commonly in place. However, some of these protocols should be evaluated for their effectiveness against non-swine origin influenza viruses (H5NX and others). Transmission of swine influenza through contaminated fomites and contaminated personnel has been documented in pigs (Allerson et al., 2013; Lopez-Moreno et al., 2023), so understanding risks from other species focusing on fomites and people is justified. Furthermore, investigations of low pathogenic avian influenza (LPAI) infections in pigs provide some clues for enhanced biosecurity programs (Abente et al., 2017; Karasin et al., 2000, 2004; Ma et al., 2007; Saavedra-Montañez et al., 2019) such as the case linked to water consumption from an adjacent lake contaminated with waterfowl feces (Karasin et al., 2000). The risk of water run-off from waste management systems from various species should be investigated as a source of potential contamination cross-over. Furthermore, conducting outbreak investigations in a systematic way of all new infections should be pursued to identify transmission pathways and biosecurity deficiencies. Outcomes of these investigations can serve in the design of feasible and comprehensive biosecurity programs to address the potential introduction of diseases through multiple routes.

Aerosol transmission and risk of exposure to workers

The role of aerosol transmission of H5N1 genotype B3.13 in dairy has been debated but there is no clear evidence that it plays a major role in H5N1 within herd transmission. Experimental and field testing have reported the detection of H5N1 in the nares of infected dairy cattle but the virus concentration is relatively low compared to that found in milk. Additionally, challenge with H5N1 in ferrets reported inefficient transmission through respiratory droplets (Eisfeld et al., 2024). However, investigations into the airborne route are not easy and are often hindered by the air sampling methods and equipment available. Air transmission could still play a meaningful role in H5N1 spread given that other factors such as herd size, animal density, housing, and ventilation conditions influence the importance of this route. H5N2 was found in high concentrations in

aerosols of turkey and layer flocks during the 2015 HPAI outbreak (Torremorell et al., 2016) and in pigs, the potential for H5N1 aerosol transmission should not be dismissed. Detection of swine influenza viruses in aerosols is well documented inside and outside barns, and aerosol exposure of farm workers is known to occur (Corzo et al., 2013; Lopez-Moreno et al., 2023; Prost et al., 2019). Aerosol transmission could still be relevant if the concentration of viral particles is low, yet animal populations are large in housing and ventilation conditions that favor aerosol exposure. Furthermore, the settling of virus-laden airborne particles on surfaces can be high if viral load in aerosols is high. Such situations could favor the transmission of H5N1 not only via aerosols but also via fomites (Garrido-Mantilla et al., 2019; Stadler et al., 2024). In addition, exposure of farm workers to contaminated aerosols as a means of occupational exposure is an additional area of concern. Swine-origin influenza virus has been detected in the nares of workers handling pigs (CDC, 2024a; Lopez-Moreno et al., 2022) and H5N1 has caused conjunctivitis, fatigue, and upper respiratory disease in poultry and dairy workers in direct contact with infected and sick animals, or during milking activities (CDC, 2024c). For these reasons, investigations into the airborne transmission of H5N1 in pigs should be made a priority in particular to assess the quantity, viability, and distribution of virus-laden airborne particles within farms and at the exhaust of ventilation systems. Furthermore, there is a need to develop guidance for personal protective equipment use for HPAI H5N1 in farm workers of the various animal species since activities and risks vary and recommendations should be adapted accordingly.

Reassortment risks

A major concern of the potential introduction of H5N1 into pigs is the risk of the virus to reassort with endemic influenzas circulating in swine. Multiple clades, clusters, and genotypes of swine influenza are circulating in pigs and pigs carry H1 and H3 influenza viruses of human origin (Anderson et al., 2021) which raises concerns of enhanced zoonotic potential for H5N1 viruses. Pigs are prone to reassort influenza viral segments when coinfected with two viral strains yielding multiple diverse viral constellations able to emerge, persist, or subside in the pigs (Li et al., 2022; Bi et al., 2024). It is of the utmost importance to monitor and surveil swine populations of pigs not only to determine the presence of H5N1 in pigs but also the type of reassortant viruses that may emerge as multiple distinct viral reassortant viruses may emerge from a single animal population. Multiple factors are involved in reassortment and on-ward transmission of reassortants, thus factors that limit pig commingling and viral replication such as vaccination and segregation should be explored to mitigate the risk of dissemination of novel reassortant viruses (Li et al., 2022; López-Valiñas et al., 2023).

Cross-protective immunity against H5N1 2.3.4.4b viruses

There is some evidence in humans that pre-existing immunity against A(H1N1)pdm09 may provide some protection against H5N1 2.3.4.4b infections due to the presence of neuraminidase

inhibition antibodies against the N1 of 2009 pandemic origin, but not prior seasonal N1 viruses (Daulagala et al., 2024; Kandeil et al., 2023). The pH1N1 derived its NA protein from the avian-origin Eurasian-avian swine viruses (Garten et al., 2009) and appears somewhat closely related to the N1 of the H5N1 virus (~89% similarity). Thus, there is some speculation whether the limited transmission documented in humans could be due to the presence of N1 reactive antibodies. Whether similar evidence is found in pigs is unknown and studies should be directed at evaluating whether there is cross-protective immunity between H5N1 2.3.4.4b viruses and N1 antibodies of pandemic, swine, and seasonal origin in pigs. Furthermore, immunity induced by H5N1 vaccines should also be assessed in pigs to further enhance the industry readiness against potential incursions of the H5N1 virus in pigs.

Diagnostics and surveillance

Current diagnostic methods routinely offered at VDLs to detect H5N1 2.3.4.4b are considered effective. These tests include rapid molecular diagnostic tests (rRT-PCR and sequencing) for the influenza A virus (IAV) matrix gene, H5 subtype, and H5 clade 2.3.4.4b. Determining influenza exposure by detecting presence of antibodies against influenza virus can be accomplished by using a nucleoprotein-based ELISA (IDEXX Laboratories, Westbrook, ME, USA) against the conserved nucleoprotein (NP) and the assay is available at most veterinary diagnostic laboratories. However, their effectiveness in surveilling H5N1-infected populations will need to be validated using the various sample types currently employed to monitor for swine influenza viruses. In part, the sensitivity and specificity of the various sample types will depend on the quality of the sample, and the viral and/or antibody load (Culhane et al., 2020; Spackman, 2020). Confirmation that commonly used sample types for detecting swine influenza viruses can work to detect H5N1 would be reassuring.

Mutations and H5N1 mammal adaptation

Some H5N1 studies have suggested mammal-to-mammal transmission (Peacock et al., 2024). Specific mutations that are thought to facilitate the virus's adaptation and dissemination have been identified in H5N1 viruses infecting several mammal species. Examples of mutations that have been observed in previous infection waves and the current H5N1 epidemic include: PB2-E627K and PB2-D701N, PB2-M631L, PB2 E627K 42 (Plaza et al., 2024; Halwe et al., 2024; Nguyen et al., 2024). The presence of these, and other mutations, needs to be monitored closely as it raises concerns for potential widespread mammal-to-mammal transmission. Should H5N1 be detected in pigs, ongoing monitoring and characterization of mutations that may suggest mammal adaptation, pig-to-pig transmission, and zoonotic potential will need to be monitored in near-real time.

H5N1 Environmental stability

Numerous studies have examined the survival and stability of influenza viruses in the environment (Spackman, 2023). The stability of avian and swine influenza virus strains remains comparable when subjected to similar conditions (Poulson et al., 2016; Spackman, 2023). The majority of studies have been conducted under experimental conditions, and comparisons between highly specific farm conditions can be challenging (Spackman, 2023). Consequently, there is a need for *in vivo/field* viability studies using clade H5N1 2.3.4.4b clade to accurately reflect the farm environment which includes multiple types of surfaces and affluents (e.g. manure, lagoon water, etc), and temperature and relative humidity conditions. Environmental stability studies should also reflect habitat for outdoor and feral pigs which are more likely to become exposed to other animals with HPAI infections.

Management practices to control H5N1 virus

Over the last few years, there has been a better understanding of within-farm swine influenza transmission which has provided the basis for improved protocols to control and eliminate endemic influenza viruses (Chamba Pardo et al., 2018; Diaz et al., 2015; White et al., 2017; Lopez-Moreno, et al., 2022; Lopez-Moreno et al., 2023). In general, there is consensus that practices that improve active and passive immunity and also decrease exposure to influenza viruses are needed to have sustainable influenza control programs. However, these protocols would need to be validated against H5N1infections in pigs and adaptations to the protocols may be needed depending on the risks and dynamics of the infections in the pigs in particular taking into consideration how well, or not, these H5N1 may be adapted in the pigs.

Lastly, animal movement is an effective pathway to disseminate viruses in pigs given the amount of pig movement that happens in the swine industry routinely (Allerson et al., 2014; Diaz et al., 2017; Nelson et al., 2015). To minimize the impact of H5N1 dissemination through animal movement, modeling efforts that assess routes and means of dissemination for other swine diseases could be adapted to H5N1. This is important because specific biocontainment strategies could be designed to prevent the impact of virus dissemination through this route.

Summary

In summary, many questions need to be answered related to H5N1 clade 2.3.4.4b risks to pigs. The questions are complex and to address them, an interdisciplinary, science-based approach will be needed to prevent the introduction of H5N1 into pigs and contain it should incursions occur. The swine industry, industries allied, governmental agencies and academia will have to work together to address unanswered questions relevant to H5N1 infections. It will take resources and a combination of experimental, field-based based, and hypothesis-driven studies to provide answers necessary for the swine industry to deal with the threat of H5N1 infections. Outcomes from these studies should focus on advancing the understanding of pathogenesis, diagnostics, transmission, epidemiology, vaccination, and biosecurity practices to help prevent and/or contain infections. Doing so effectively, will not only protect and improve the health of pigs but also will ensure food security, and sustainable food production systems and will prevent infections in people.

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