In the spring of 2013, human infections with novel avian-origin H7N9 influenza viruses were reported in China (1), marking the first time this subtype has appeared in humans. One hundred thirty-four human infections have been confirmed, with a resulting mortality rate of approximately 32% (2–4). Identification of the source of the viruses is under investigation, but exposure to infected domestic poultry has been indicated in the majority of the cases. At present, human-to-human transmission is unsustained (5).

The H7N9 viruses contain genes solely of avian origin, with surface proteins donated from ducks or other aquatic waterfowl and internal proteins from chickens or other migratory wild birds (2, 6). Currently, there are two lineages of H7N9 viruses reported, with A/Anhui/1/13 (Anhui/1)-like viruses possessing molecular markers associated with adaptation to humans: mutations in the receptor binding hemagglutinin (HA) protein that confer enhanced binding to mammalian receptors (S138A, G186V, and Q226L [H3 numbering]) and the E627K mutation in PB2 that enhance binding to mammalian receptors (S138A, G186V, and Q226L [H3 numbering]). These mutations may enhance the ability of H7N9 viruses to infect humans, and the potential that they may infect swine and generate additional mammalian adaptations is a significant public health concern. Recent studies by Zhu et al. demonstrated that a single human H7N9 isolate could infect pigs experimentally but could not be transmitted to cage mates (11). For further risk assessment and understanding of the pathogenesis of H7N9 viruses in swine, evaluation of additional human H7N9 isolates is warranted. However, studies that involve a large animal model, such as swine, can be cumbersome and expensive. Recently, models have been developed for culture and infection of swine respiratory tissues ex vivo. Importantly, these respiratory tissue explants retain their structure and receptor specificity and mimic the infection patterns observed for live animals (12–14). Furthermore, these ex vivo studies provide for a rapid and humane option to test larger numbers of influenza virus isolates to preselect virus candidates or to use as a substitute for live-animal studies.

Recently, novel H7N9 influenza viruses have caused an unprecedented outbreak in humans. Pigs are an important intermediate host for influenza; thus, we assessed the replication ability of three human H7N9 viruses (A/Anhui/1/2013, A/Shanghai/1/2013, and A/Shanghai/2/2013) in swine tissue explants. All viruses tested replicated efficiently in explants from tracheas and bronchi, with limited replication in alveolar cells. Swine respiratory tissue explants can serve as an efficient model for screening replication potential of newly emerging H7N9 viruses.
every 3 h, with a total three changes, in order to remove cytokines, and the explants were incubated at 37°C in 5% CO2 for 18 h before inoculation. The explants were washed extensively with phosphate-buffered saline (PBS) prior to inoculation with 10^6 EID50 units/explant. After a 1-h adsorption, the explants were washed 2 times with 0.9% NaCl, pH 2.2, and 3 times with PBS. For each time point indicated (see Fig. 1), 300 µl of infection medium (BEBM plus 0.5% bovine serum albumin) was added to the apical surface for 30 min, harvested, and titrated on MDCKs, with the 50% tissue culture infectious dose (TCID50) determined using the method of Reed and Muench (17). Tissue samples were obtained every 24 h for 3 days. Data are representative of results of 3 or 4 independent experiments with two or more explants per tissue and per virus.

As expected, Swine/MO was able to productively replicate in both tracheal and lung explants (Fig. 1A and B). The negative-control avian virus, Duck/NJ, failed to replicate in either tissue at any time point sampled. The three human H7N9 viruses tested replicated efficiently in both tracheal and lung explants, with the highest virus titers detected at 48 or 72 hpi inoculated with human H7N9 Anhui/1 (A and B), Shang/1 (C and D), Shang/2 (E and F), positive-control Swine/MO (G and H), and negative-control Duck/NJ (I and J) influenza viruses were analyzed. Tissues were fixed and stained for influenza A virus nucleoprotein (brown) and imaged at an objective magnification of ×10 (lung) or ×20 (trachea). In tracheal explants, influenza A viral antigen-positive cells (black arrows) were diffusely spread in tracheal epithelium inoculated with H7N9 viruses (A and C) and in the swine virus control (G) and as multiple foci in Shang/2 influenza virus-inoculated samples (E). Antigen-positive cells were also found as a continuous line in bronchial epithelium (red arrows) inoculated with H7N9 viruses (B, D, and F) and in the swine virus control (H) and as single alveolar cells (B, D, and H) in lung explants. Antigen-positive cells were limited to the bronchial epithelium in lung explants inoculated with Shang/2 (F).

All human H7N9 viruses and Swine/MO-infected tissues displayed positive influenza nucleoprotein (NP) antigen staining as expected. NP staining was detected in tracheal epithelia inoculated with influenza viruses Anhui/1, Shang/1, Shang/2, and Swine/MO (Fig. 2). In lung explants, viral antigen staining was largely restricted to the bronchi, with the exception of Anhui/1, Shang/2 and Swine/MO, for which limited positive viral antigen staining was detected in cells of the alveoli adjacent to infected bronchi. In contrast, no viral antigen staining was detected in either tracheal or lung explants inoculated with avian influenza Duck/NJ virus.

Several factors contribute to the important role that swine play in the genesis and dissemination of novel influenza viruses: (i) both human-like α2,6- and avian-like α2,3-linked sialosides are present in the porcine respiratory tract, (ii) experimental studies have confirmed the susceptibility of pigs to both avian and human influenza viruses (12), and (iii) pigs commonly interact with both humans and avian species in agricultural settings. The emergence of avian H7N9 viruses that are capable of infecting humans and that possess multiple molecular markers associated with adapta-
tion to mammals is unprecedented and troubling. To date, there is little evidence of human-to-human transmission of H7N9; however, the potential circulation of this virus in pigs may provide critical changes that alter pathogenicity or transmission potential. In this study, we build upon in vivo observations from Zhu et al., who demonstrated a single H7N9 isolate (A/Shanghai/2/13) is able to replicate in pigs with peak titers ranging between 3.5 and 5.6 \( \log_{10} \) TCID\(_{50} \)/ml (11). We have demonstrated ex vivo that three human H7N9 viruses, including the genetically distinct Shang/1 virus, are able to replicate in both the upper and lower respiratory tracts of pigs, with peak titers ranging from 6.0 to 7.2 \( \log_{10} \) TCID\(_{50} \)/ml. The slightly higher titers seen with the explants than those seen in vivo (11) may be due to differences in relative inoculum and tissue size between the ex vivo model and a live pig. However, lowering the starting inoculum by multiple logs (10\(^4\) EID\(_{50}\) units/explant) yields similar infectious titers and growth kinetics (data not shown).

This study confirms that recently emerged H7N9 viruses are capable of infecting pigs, consistent with the work of others (11, 18). This significantly increases the risk associated with H7N9 viruses and their potential for acquisition of mutations or gene segments that could enhance transmissibility in mammals. Confirmed human infections in July 2013 support the idea that these viruses are still at large in the field (4), but currently, no systematic swine H7N9 influenza virus surveillance is performed in Northern or Eastern China. The data from this study validates the swine explant model for screening the replication potential of H7N9 viruses in porcine tissues and, more broadly, indicates that this model will be valuable for screening the host range and pathogenicity of emerging influenza viruses before proceeding with live-animal models.

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