

LETTER TO THE EDITOR

No influenza D virus detected among pigs, northern Vietnam

Dear Editor Cowling, In 2011, influenza D virus (IDV) was first isolated from US pigs that were exhibiting influenza-like illness.^{1,2} Though it was initially classified as a subtype of influenza C virus, IDV is now recognized as a novel member of the Orthomyxoviridae family of viruses.³ Since its discovery in pigs, IDV has been isolated in a number of other animal species including cattle, horses, sheep, goat, and camelids. Most recently, we found evidence of IDV in poultry in Sarawak, Malaysia.⁴

The zoonotic potential of IDV has not been extensively studied, and there are currently no known cases of IDV transmission among humans. Interestingly however, a 2015 study confirmed that bovine IDV can be replicated and transmitted among guinea pigs and ferrets, which are a model for human influenza virus infection.⁵ Additionally, in 2016, a cross-sectional serological study conducted in Florida detected a marked increase in anti-IDV antibodies among cattle workers compared to non-cattle-exposed individuals.⁶ Together, these studies suggest that IDV could be an emerging zoonotic threat.

Globally, IDV has also been identified in China,⁷ Japan,⁸ France,⁹ Italy,¹⁰ Argentina,¹¹ Turkey,¹² Kenya, Morocco, Togo, and Benin.¹³ A high density of animal farms and markets, limited public health

resources, and variation in biosecurity measures make Vietnam an ideal location for the propagation of novel zoonoses like IDV. However, currently we know of no published literature on IDV surveillance among domesticated animals in Vietnam. As such, the primary aim of this investigation was to determine the prevalence of IDV among pigs in northern Vietnam.

From May 2019 to February 2020, as part of an ongoing influenza A virus surveillance study, we collected samples from five swine farms across northern Vietnam (Figure 1). The resultant 823 samples included bioaerosol samples (91, 11%), fecal samples (272, 33%), swine oral secretions (276, 34%), and farmworker nasal washes (184, 22%). The bioaerosol samples were collected using National Institute of Biosafety and Health's (NIOSH) model BC251 two-stage bioaerosol samplers. The samplers were affixed to a stationary tripod, calibrated to a rate of 3.5 L/min, and placed in pig pens for 3 hours. FLOQSwabs (Copan Diagnostics) were used to collect the fecal samples from pig enclosures. Swine oral secretions were collected by fixing cotton ropes at animal height for chewing. Once the ropes were sufficiently chewed—for a period of approximately 30–45 minutes—the fluid was extracted from the rope into sterile



FIGURE 1 Geographic distribution of sampling locations (black stars). Samples were collected between May 2019 and February 2020 from the two farms in the Lao Cai province, two in Bac Giang, and one in Quang Ninh

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cryovials. Additionally, after appropriate consent, nasal washes were collected from farm employees by a trained assistant who injected 5 mL of sterile water into one nostril and collected the expressed fluid in a sterile specimen cup. All samples were labeled appropriately and stored at -80°C until RNA extraction was performed.

QIAamp Viral RNA Mini Kits (Qiagen) were used for RNA extraction. Viral RNA extracts were analyzed via quantitative real-time polymerase chain reaction (qRT-PCR) using Superscript III One-Step RT-PCR System with platinum (Thermo Fisher Scientific, Inc) and influenza D virus-specific primers and probes.¹ Synthetic positive and negative controls were used in each PCR run.

Influenza D virus was not detected in any of the samples. As prevalence among pigs has been high in other countries, this finding was unexpected. This might be explained in several ways. Perhaps there is no influenza D in these farms. We have also had difficulty detecting influenza A in these farms but, using the same assays and laboratorians, found a high prevalence of influenza A among live bird markets in the same geographical areas.¹⁴ It is also possible that there was an inherently low prevalence of IDV on the selected farms, most of which were confined to northern areas, larger and industrialized with solid biosecurity protocols in place. The inclusion of more farms, especially smaller, community farms, in other provinces might reverse our molecular influenza A observations.

Despite the limitations of geographical area and scale, our study is valuable as it is the first to assess IDV prevalence in Vietnam. A major strength of our study is the use of bioaerosol sampling technique which has been success for a number of viral surveillance studies. Future epidemiological investigations should be done to further characterize the prevalence of IDV in other regions of Vietnam and in other countries. Such studies of IDV will be essential for our understanding of its zoonotic potential and could impact biosecurity measures, such as use of personal protective equipment, on animal farms.

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CONFLICT OF INTEREST

None declared.



AUTHOR CONTRIBUTIONS

Amanda S Farrell: Conceptualization (equal); Investigation (equal); Writing-original draft (lead); Writing-review & editing (supporting).
Vuong Nghia Bui: Conceptualization (equal); Investigation (supporting);

Methodology (supporting); Project administration (equal); Resources (equal); Supervision (lead); Writing-review & editing (supporting).
Tung Duy Dao: Investigation (equal); Methodology (supporting); Project administration (equal); Supervision (supporting); Writing-review & editing (supporting).
Trung Duc Hoang: Investigation (equal); Methodology (supporting).
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