

Avian influenza virus surveillance in live bird markets, northern Vietnam

Authors

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Abstract

Background: We sought to employ a One Health approach in examining live bird markets in northern Vietnam for evidence of avian influenza A viruses.

Methods: Environmental, animal, and animal worker specimens were collected from four live bird markets (LBMs) across provinces (Hanoi, Lang Son, Lao Cai, and Quang Ninh) in northern Vietnam from October 2019 – August 2020. At each sampling visit, up to three NIOSH two-stage aerosol samplers were assembled on tripods, positioned 0.5 m from the ground, and run at 3.5 L/min for up to 4 hrs. Up to ten oropharyngeal (OP) and fifteen cage swabs were collected from poultry found near the NIOSH samplers. Market poultry workers completed questionnaires and permitted nasal wash specimen collections. Specimens were screened for influenza A virus using real-time reverse transcription polymerase chain reaction (qRT-PCR). Samples found to be positive were then used for egg inoculation to assess viability of the detected virus.

Results: 1296 LBM samples were collected. Among the 1058 LBM samples examined by qRT-PCR thus far, 333 (31.5%) were positive for influenza A virus. Among the 717 samples examined with egg culture, 173 (24.1%) have yielded viable influenza A virus; these include 22 bioaerosol samples, 81 poultry cage swabs, 70 poultry OP swabs, and 0 human nasal washes (Table 1).

Conclusion: Poultry cages and bioaerosols are sources of infectious influenza A virus in LBMs in northern Vietnam and may pose an occupational risk of infection as evidenced by virus-positive human nasal washes. Additionally, bioaerosol sampling is a sufficient influenza A virus surveillance tool that could potentially replace more invasive bird swab sampling.

Materials and Methods

Sample Collection: At each sampling visit, up to three NIOSH two-stage aerosol samplers were assembled on a tripod and positioned 0.5 m from the ground in poultry-dense sections of the market (Figure 1). Samplers were outfitted with a 15 ml Falcon tube, 1.5 ml centrifuge tube, and a polytetrafluoroethylene (PTFE) filter designed to collect viral particles. SKC AirChek Touch pumps (SKC, Eighty Four, Pennsylvania) were connected to the NIOSH samplers and ran for four hours collecting air at 3.5 L/min. Up to ten oropharyngeal (OP) and fifteen cage swabs were collected from poultry found near the NIOSH aerosol samplers, with preferential selection for any birds that appeared sick. Swabs were placed individually into sterile tubes containing 2.5 ml of viral transport medium. Market poultry workers were asked to permit the collection of one nasal wash. Participants were directed to tilt their heads back at a 70-degree angle, holding their breath as one nostril was irrigated with a teaspoon (5 ml) of sterile water using a 10 ml plastic syringe. The participant then tilted their head back down, releasing the fluid into a sterile specimen collection cup. These workers were additionally asked to complete a brief questionnaire regarding potential exposure to and knowledge of avian influenzas. Following initial study enrolment, workers were able to participate across subsequent visits.

Materials and Methods

Sample Processing: All samples were stored on ice for transfer to the central laboratory at the National Institute of Veterinary Research in Hanoi, Vietnam. Upon arrival, samples were either processed immediately or preserved at -80° C.

Bioaerosol samples

NIOSH samplers were disassembled in the laboratory for immediate processing. Sterile virus collection medium (phosphate buffered saline with bovine serum albumin fraction V) were added to both the 15 ml Falcon tube and 1.5 ml centrifuge tube, after which each was vortexed thoroughly prior to transferring the liquid wash to individual 2.0 ml cryovial tubes. The PTFE filter was then removed from its casing and dry vortexed for 15 seconds in a 50 ml Falcon tube. Next, virus collection media was added to the tube containing the filter, taking care to wet the filter. After thorough vortexing, the filter was discarded, and the remaining media was pooled with the sample collected from the corresponding 1.5 ml centrifuge tube.

Swab samples and washes

Tubes containing collected swabs were vortexed at medium speed. Swabs were then discarded, and the remaining viral transport media was transferred to 2.0 ml cryovials. Worker nasal wash samples required no additional processing prior to molecular work.

Sample Analysis: Viral RNA was extracted from processed samples using the QIAamp Viral RNA Mini Kit (Qiagen). Sample RNA was screened for influenza A virus using qRT-PCR. Positive samples were then used for egg inoculation to assess viability of the detected virus.

Figure 1. NIOSH BC 251 sampler on a tripod in a LBM, and sampler schematic



Results

From October 2019 – August 2020, 1296 LBM samples were collected. Among the 1058 LBM samples examined by qRT-PCR thus far, 333 (31.5%) were positive for influenza A virus. Among the 717 samples examined with egg culture, 173 (24.1%) have yielded viable influenza A virus; these include 22 bioaerosol samples, 81 poultry cage swabs, 70 poultry OP swabs, and 0 human nasal washes (Table 1).

Table 1. Summary of field and laboratory results from live bird market (LBM) samples collected in northern Vietnam, October 2019 – August 2020

Sample types	Number collected	Molecular screening		Egg culture screening	
		Number of specimens examined with qRT-PCR	Among specimens studied, percent positive for influenza A virus by qRT-PCR	Number of specimens examined with egg culture	Among specimens studied, percent to yield influenza A virus from egg culture
Human nasal washes	324	270	2.9%	180	0.0%
Poultry oropharyngeal swabs	324	270	45.2%	180	38.9%
Poultry cage swabs	486	405	36.5%	270	30.0%
Bioaerosol	162	135	40.7%	87	25.3%

Conclusion

Poultry cages and bioaerosols are sources of infectious influenza A virus in LBMs and may pose an occupational risk of infection as evidenced by virus-positive human nasal washes. Additionally, bioaerosol sampling is a sufficient influenza A virus surveillance tool that could potentially replace more invasive bird swab sampling.