HAL open science

Aerosol exposure of live bird market workers to viable influenza A/ H5N1 and A/ H9N2 viruses, Cambodia

Paul Horwood, Srey Viseth Horm, Sokhoun Yann, Songha Tok, Malen Chan, Annika Suttie, Phalla Y, Sareth Rith, Jurre Siegers, Sorn San, et al.

To cite this version:

Paul Horwood, Srey Viseth Horm, Sokhoun Yann, Songha Tok, Malen Chan, et al.. Aerosol exposure of live bird market workers to viable influenza A/ H5N1 and A/ H9N2 viruses, Cambodia. Zoonoses and Public Health, 2023, 70 (2), pp.171-175. 10.1111/zph.13009 . pasteur-04096779

HAL Id: pasteur-04096779

https://riip.hal.science/pasteur-04096779v1

Submitted on 13 May 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License

Check for updates

Received: 4 September 2022 | Revised: 18 October 2022 | Accepted: 7 November 2022

DOI: 10.1111/zph.13009

SHORT COMMUNICATION

WILEY

Aerosol exposure of live bird market workers to viable influenza A/H5N1 and A/H9N2 viruses, Cambodia

Paul F. Horwood[1,2] | Srey Viseth Horm[1] | Sokhoun Yann[1] | Songha Tok[1] | Malen Chan[3] | Annika Suttie[1,4] | Phalla Y[1] | Sareth Rith[1] | Jurre Y. Siegers[1] | Sorn San[5] | Holl Davun[5] | Sothyra Tum[5] | Sowath Ly[3] | Arnaud Tarantola[3] | Philippe Dussart[1] | Erik A. Karlsson[1]

[1]Virology Unit, Institut Pasteur du Cambodge, Pasteur Network, Phnom Penh, Cambodia

[2]College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia

[3]Epidemiology and Public Health Unit, Institut Pasteur du Cambodge, Pasteur Network, Phnom Penh, Cambodia

[4]School of Applied and Biomedical Sciences, Federation University Australia, Churchill, Victoria, Australia

[5]National Animal Health and Production Research Institute, Cambodian Ministry of Agriculture, Forestry and Fisheries, Phnom Penh, Cambodia

Correspondence
Paul F. Horwood, Australian Institute of Tropical Health and Medicine, James Cook University, PO Box 6811, Cairns, QLD 4870, Australia.
Email: paul.horwood@jcu.edu.au

Erik Karlsson A, Virology Unit, Institut Pasteur du Cambodge, 5 Monivong Blvd, PO Box 983, Phnom Penh, Cambodia.
Email: ekarlsson@pasteur-kh.org

Present address
Arnaud Tarantola, Regional Epidemiology Unit, Santé Publique France, Paris, France

Philippe Dussart, Institut Pasteur de Madagascar, Pasteur Network, Antananarivo, Madagascar

Funding information
Food and Agriculture Organization of the United Nations; National Institute of Allergy and Infectious Diseases; US Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response

Abstract

Live bird markets (LBMs) have been identified as key factors in the spread, persistence and evolution of avian influenza viruses (AIVs). In addition, these settings have been associated with human infections with AIVs of pandemic concern. Exposure to aerosolised AIVs by workers in a Cambodian LBM was assessed using aerosol impact samplers. LBM vendors were asked to wear an air sampler for 30 min per day for 1 week while continuing their usual activities in the LBM during a period of high AIV circulation (February) and a period of low circulation (May). During the period of high circulation, AIV RNA was detected from 100% of the air samplers using molecular methods and viable AIV (A/H5N1 and/or A/H9N2) was isolated from 50% of air samplers following inoculation into embryonated chicken eggs. In contrast, AIV was not detected by molecular methods or successfully isolated during the period of low circulation. This study demonstrates the increased risk of aerosol exposure of LBM workers to AIVs during periods of high circulation and highlights the need for interventions during these high-risk periods. Novel approaches, such as environmental sampling, should be further explored at key high-risk interfaces as a potentially cost-effective alternative for monitoring pandemic threats.

KEYWORDS
A/H5N1, A/H9N2, aerosol, avian, influenza, live bird market

Senior authors Philippe Dussart and Erik A Karlsson contributed equally to this article.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2022 The Authors. *Zoonoses and Public Health* published by Wiley-VCH GmbH.

1 | INTRODUCTION

Live bird markets (LBMs) are major hubs for poultry commerce throughout the world. Commonly, a variety of birds enter LBMs through the poultry value chain, including from backyard and semi-commercial producers. Close contact between birds of many species from different sources leads to infection and amplification of numerous pathogens, including avian influenza viruses (AIVs). These pathogens then persist in the LBM environment due to insufficient biosecurity measures. As such, LBMs provide numerous interfaces for human exposure to AIVs, including contact with the contaminated market environment, direct contact with poultry or poultry products, and inhalation of viral particles suspended in the air column (Amonsin et al., 2008; Indriani et al., 2010; Vergne et al., 2019; Wang et al., 2006). Most LBM surveillance systems focus on detection of viruses in the birds themselves. However, it is more likely that LBM workers will be infected by aerosolized viruses in the market environment. Limited studies have been conducted to investigate this route of exposure including comparing environmental/animal samples collected concurrently and studies to estimate the risk of human infection (Bui et al., 2019; Zhou et al., 2016).

Previous longitudinal studies in Cambodian LBMs have documented a consistent pattern of AIV circulation, with peak viral activity occurring during festival periods, especially between January–March and October–November. The period of lowest circulation occurs at midyear (Horm et al., 2011, 2016; Horwood et al., 2018; Karlsson et al., 2019). To examine how AIV circulation in poultry compared with the presence of AIVs in the LBM air column and assess the risk of LBM workers inhaling live virus during their routine work activities, we collected concurrent poultry, wash water and air samples during periods of high and low viral circulation.

Impacts

- We determined that avian influenza viruses of public health importance (A/H5N1 and A/H9N2) could be detected in the air column of a live bird market during a period of known high circulation.
- Testing showed that many of the viruses detected in the study were infectious, thus highlighting the risk of aerosol exposure to avian influenza viruses in live bird markets.
- Further research is needed to better understand the routes of exposure for zoonotic pathogens at high-risk interfaces and to determine if interventions during periods of high circulation can reduce the risk of zoonotic transmission.

2 | MATERIALS AND METHODS

Samples were collected from a central LBM in Phnom Penh, Cambodia, for five consecutive days during known periods of peak and low AIV circulation, February 22–26 and May 23–27, 2016, respectively. Oropharyngeal and cloacal swabs (pooled for each animal) were concurrently collected from four ducks and chickens selected randomly from throughout the LBM each day. Two 50 ml samples of carcass wash water (water used to wash poultry carcasses once slaughtered and defeathered) were also collected from two randomly selected containers in the market each day. To test the LBM air, two LBM vendors agreed to wear a Sioutas Personal Cascade Impactor Sampler (SKC Inc.) each day of collection for 30 min while continuing with their usual activities in the LBM (Figure 1). The pump unit (Leland Legacy pump: SKC Inc.) was operated at a flow rate of 9 L/min to simulate normal human breathing. The impact chamber contained five collection stages of polytetrafluoroethylene filters (SKC Inc.) with size cutoff values of (A) 2.5–10 μM; (B) 1–2.5 μM; (C) 0.5–1 μM; (D) 0.25–0.5 μM; and (E) <0.25 μM. These methods have been described previously for the detection of aerosolised influenza A/H3N2 in an indoor setting (Lednicky & Loeb, 2013). Following 30 min of wearing the impact sampler, the filter unit was immediately transported to the laboratory in an ice box to reduce inactivation of the viruses due to desiccation. Individual filters were then cut into three pieces, using sterile techniques, and placed in 3 ml of viral transport media (VTM). The VTM tube was vortexed to assist in removing material from the filters, split into three aliquots and stored at −80°C until required for testing.

All samples were processed according to protocols detailed previously (Horm et al., 2016; Horwood et al., 2018; Karlsson et al., 2019). Briefly, nucleic acids were extracted from all samples (processed air filters, wash water samples and chicken/duck swabs) using the QIAamp Viral RNA Mini Kit (QIAGEN), according to the manufacturer's instructions. Extracts were then tested for influenza matrix (M) gene, H5 (H5a and H5b primer sets), H7, and H9 by real-time RT-PCR. All assays were sourced from the International Reagent Resource (https://www.internationalreagentresource.org/). Samples that tested positive for influenza A virus were inoculated into specific pathogen-free embryonated chicken eggs using standard methods (Horm et al., 2012).

Ethical approval for this study was granted by the Cambodian National Ethics Committee for Human Research (approval No. 051NECHR). Animal sampling was conducted by the National Animal Health and Production Research Institute (NAHPRI) under the direction of the General Directorate for Animal Health and Production, Cambodian Ministry of Agriculture, Forestry and Fisheries as part of routine disease surveillance activities.

3 | RESULTS

Throughout the 2 weeks of collection, 20 LBM vendors (10 vendors per period) wore the air filter apparatus for 30 min while conducting their usual activities. AIV was detected by real-time RT-PCR in the filter apparatus significantly more frequently during the peak season in February (100%) compared with the low period in May (0%). Influenza A/H5 and A/H9 were detected in 40% and 50% of the air

18632378, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/zph.13009 by Institut Pasteur, Wiley Online Library on [16/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

FIGURE 1 A live bird market vendor wearing a personal aerosol impact sampler during the study

TABLE 1 Summary results from samples collected from a Cambodian live bird market during peak (February) and low (May) periods of avian influenza circulation

	Detection and isolation of avian influenza viruses				
	M-gene[a]	H5 (H5a/H5b)[a]	H7[a]	H9[a]	Isolation[b]
Peak period (Feb)					
Aerosol (n = 10)	10 (100%)	4 (40%)	0	5 (50%)	H5N1: 5 (50%); H9N2: 2 (20%)
Chicken (n = 20)	14 (70%)	6 (30%)	0 (0%)	6 (30%)	ND
Duck (n = 20)	15 (75%)	8 (40%)	0 (0%)	4 (20%)	ND
Wash water (n = 10)	10 (100%)	ND	ND	ND	ND
Low period (May)					
Aerosol (n = 10)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Chicken (n = 20)	10 (50%)	0 (0%)	0 (0%)	6 (30%)	ND
Duck (n = 20)	3 (15%)	1 (5%)	0 (0%)	2 (10%)	ND
Wash water (n = 10)	10 (100%)	ND	ND	ND	ND

[a]Avian influenza detection was conducted using real-time RT-PCR using assays sourced from the International Reagent Resource (https://www.internationalreagentresource.org/Home.aspx).

[b]Isolation of avian influenza viruses was attempted using embryonated chicken eggs.

samples collected during the peak period of circulation, respectively (Table 1). No patterns were observed that suggested AIVs were detected more frequently in the larger or smaller pore size filters (Table S1).

Live virus was recovered by egg-based amplification from the air filter apparatus of 50% of participants from samples collected during peak AIV circulation (February). Influenza A/H5N1 was recovered from 5 (50%) of the participant samples collected during this period; and in 2 (20%) of these samples A/H9N2 was also concurrently isolated. Live virus was not isolated from any of the samples during the period of low circulation (May).

During the same 2 weeks of sampling (1 week during high and low AIV circulation, respectively), 40 chicken samples, 40 duck samples and 20 wash water samples were also collected. During February, AIV RNA was detected in 70%, 75%, and 100% of chicken, duck, and wash water samples, respectively. In May, AIV RNA was detected in 50%, 15% and 100% of chicken, duck and wash water samples, respectively. A/H5 subtype RNA was detected in 30% of chicken and 40% of duck samples during the peak period, compared with 0% and 5% during the low period, respectively. A/H9 subtype RNA was detected in 30% of chicken and 20% of duck samples during the peak period, compared with 30% and 10% during the low period, respectively. Influenza A/H7 RNA was not detected in any of the air, bird, or environmental samples throughout the study (Table 1).

Sequence analysis of the hemagglutinin (HA) and neuraminidase (NA) genes of several A/H5N1 isolates using Sanger methodology revealed the influenza A/H5N1 viruses to be typical of other clade 2.3.2.1c viruses circulating in LBMs during 2015–2016 (Suttie

18632378, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/zph.13009 by Institut Pasteur, Wiley Online Library on [16/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

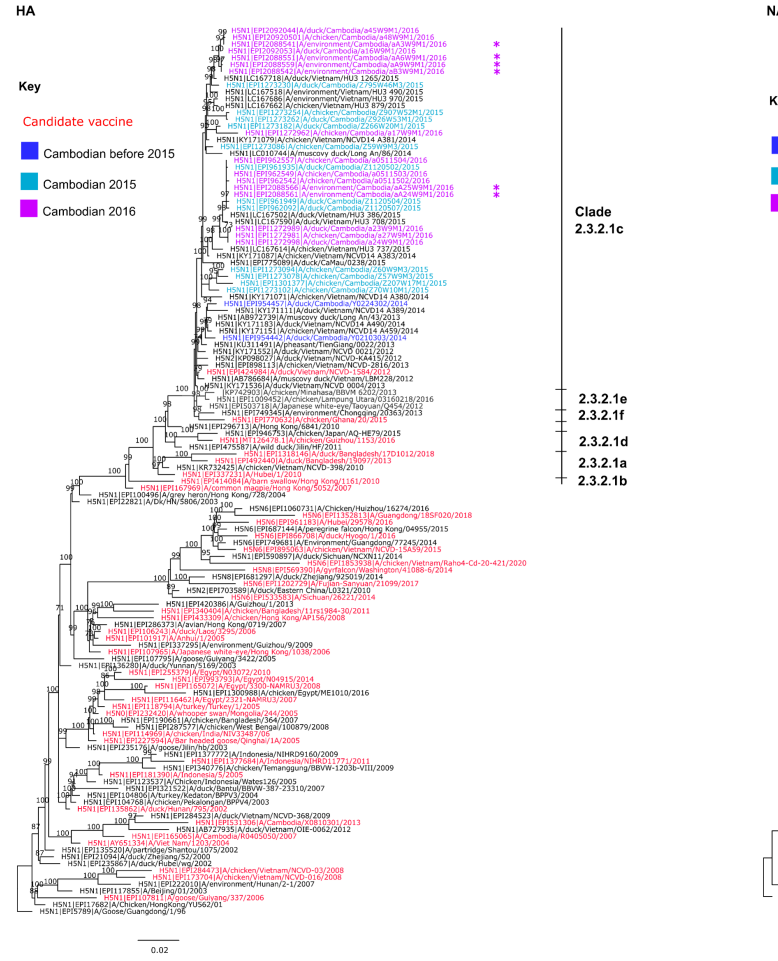

FIGURE 2 Maximum likelihood phylogeny of the hemagglutinin (HA) and neuraminidase (NA) genes of A/H5N1 viruses detected in air samples in a Cambodian live bird market, 2016. (A) HA and (B) NA of A/H5N1 clade 2.3.2.1c viruses in air and poultry samples. Trees were generated with IQ-tree using the TVM+V+I+G4 and GTR+F+G4 model, respectively. Phylogenetic support was estimated using 1000 ultrafast bootstrap replicates. Cambodian isolates are shown before 2015 (dark blue), during 2015 (teal) and in 2016 (pink). Air samples from this study are indicated with a pink star. Candidate vaccine viruses are indicated in red. Bootstrap values are indicated next to significant nodes

et al., 2019). Comparison of HA and NA sequences from air and poultry samples shows direct correlation, indicating that poultry within the LBM were the source of the viruses in the air (Figure 2).

4 | DISCUSSION

Live bird markets have been identified as key locations for zoonotic infections with AIVs such as A/H5N1 and A/H7N9 (Cowling et al., 2013). In these environments, viruses may be aerosolised by a number of means including defeathering poultry (manual or mechanized) or suspended in dust from faeces or feather follicles. A range of influenza viruses have previously been detected through aerosol collection from LBMs in Asia, including A/H7N9, A/H9N2 and A/H5N6 (Bui et al., 2019; Wang et al., 2020; Wu et al., 2017, 2018; Zeng et al., 2017; Zhou et al., 2016). The high rate of detection during the peak period of circulation is very concerning for the risk of infection in susceptible individuals.

Avian influenza virus detection in poultry and wash water correlated with previous studies, with considerably higher rates of AIV positivity in February compared with May. This study demonstrates the increased risk of aerosol exposure of LBM workers to viable AIVs during periods of high circulation, and highlights the need for more research and risk-reduction interventions during these high-risk periods. Novel methods for monitoring pathogen circulation in high-risk interfaces, such as environmental sampling, need to be further explored to identify improved approaches to surveil for pandemic threats. Environmental sampling presents an attractive alternative (or complement) to traditional sampling of individual animals. The collection of environmental samples is non-invasive, non-intrusive and is well accepted by market vendors. These approaches have the potential to provide longitudinal monitoring of live animal markets and are conducive to automation, potentially with the use of metagenomic analysis for unbiased monitoring of emerging pathogens. Further studies are needed to determine the optimal sampling frames to ensure high sensitivity and low cost.

ACKNOWLEDGEMENTS

We thank the vendors from the participating live bird markets for their cooperation with our study team. We also thank the technical

18632378, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/zph.13009 by Institut Pasteur, Wiley Online Library on [16/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

team from the Virology Unit at the Institut Pasteur du Cambodge and the field teams from the National Animal Health and Production Research Institute (Cambodian Ministry of Agriculture, Forestry and Fisheries) and the Epidemiology and Public Health Unit at the Institut Pasteur du Cambodge. This study was funded by the US Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response (grant No. IDSEP 140020-01-00; https://www.asideproject.org/). E.A.K and further work at Institut Pasteur du Cambodge is supported, in part, by federal funds from the Food and Agriculture Organization of the United Nations, the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services Contract No. 1U01AI151758-01. Open access publishing facilitated by James Cook University, as part of the Wiley - James Cook University agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

ORCID

Paul F. Horwood https://orcid.org/0000-0001-9622-0742
Philippe Dussart https://orcid.org/0000-0002-1931-3037

REFERENCES

Amonsin, A., Choatrakol, C., Lapkuntod, J., Tantilertcharoen, R., Thanawongnuwech, R., Suradhat, S., Suwannakarn, K., Theamboonlers, A., & Poovorawan, Y. (2008). Influenza virus (H5N1) in live bird markets and food markets, Thailand. *Emerging Infectious Diseases.*, *14*, 1739–1742.

Bui, V. N., Nguyen, T. T., Nguyen-Viet, H., Bui, A. N., McCallion, K. A., Lee, H. S., Than, S. T., Coleman, K. K., & Gray, G. C. (2019). Bioaerosol sampling to detect avian influenza virus in Hanoi's largest live poultry market. *Clinical Infectious Diseases.*, *68*, 972–975.

Cowling, B. J., Jin, L., Lau, E. H., Liao, Q., Wu, P., Jiang, H., Tsang, T. K., Zheng, J., Fang, V. J., Chang, Z., Ni, M. Y., Zhang, Q., Ip, D. K. M., Yu, J., Li, Y., Wang, L., Tu, W., Meng, L., Wu, J. T., … Yu, H. (2013). Comparative epidemiology of human infections with avian influenza a H7N9 and H5N1 viruses in China: A population-based study of laboratory-confirmed cases. *The Lancet.*, *382*, 129–137.

Horm, S. V., Gutiérrez, R. A., Sorn, S., & Buchy, P. (2012). Environment: A potential source of animal and human infection with influenza a (H5N1) virus. *Influenza and Other Respiratory Viruses.*, *6*, 442–448.

Horm, S. V., Sorn, S., Allal, L., & Buchy, P. (2011). Influenza a(H5N1) virus surveillance at live poultry markets, Cambodia, 2011. *Emerging Infectious Diseases.*, *19*, 305–308.

Horm, S. V., Tarantola, A., Rith, S., Ly, S., Gambaretti, J., Duong, V., Sorn, S., Holl, D., Allal, L., Kalpravidh, W., Dussart, P., Horwood, P. F., & Buchy, P. (2016). Intense circulation of a/H5N1 and other avian influenza viruses in Cambodian live-bird markets with serological evidence of sub-clinical human infections. *Emerging Microbes & Infections.*, *5*, e70 1, 9.

Horwood, P. F., Horm, S. V., Suttie, A., Thet, S., Y, P., Rith, S., Sorn, S., Holl, D., Tum, S., Ly, S., Karlsson, E., Tarantola, A., & Dussart, P. (2018). Co-circulation of influenza a/H5N1 with H7 and H9 viruses in Cambodian live bird markets with evidence of frequent co-infections in poultry. *Emerging Infectious Diseases.*, *24*, 352–355.

Indriani, R., Samaan, G., Gultom, A., Loth, L., Indryani, S., Adjid, R., Dharmayanti, N. L., Weaver, J., Mumford, E., Lokuge, K., Kelly, P. M., & Darminto. (2010). Environmental sampling for avian influenza virus a (H5N1) in live-bird markets, Indonesia. *Emerging Infectious Diseases.*, *16*, 1889–1895.

Karlsson, E. A., Horm, S. V., Tok, S., Tum, S., Kalpravidh, W., Claes, F., Osbjer, K., & Dussart, P. (2019). Avian influenza virus detection, temporality and co-infection in poultry in Cambodian border provinces, 2017–2018. *Emerging Microbes & Infections.*, *8*, 637–639.

Lednicky, J. A., & Loeb, J. C. (2013). Detection and isolation of airborne influenza a H3N2 virus using a Sioutas personal Cascade impactor sampler. *Influenza Research and Treatment.*, *2013*, 656825 1, 8.

Suttie, A., Tok, S., Yann, S., Keo, P., Horm, S. V., Roe, M., Kaye, M., Sorn, S., Holl, D., Tum, S., Buchy, P., Barr, I., Hurt, A., Greenhill, A. R., Karlsson, E. A., Vijaykrishna, D., Deng, Y. M., Dussart, P., & Horwood, P. F. (2019). Diversity of a(H5N1) clade 2.3.2.1c avian influenza viruses with evidence of reassortment in Cambodia, 2014-2016. *PLoS One*, *14*, e0226108.

Vergne, T., Meyer, A., Long, P. T., Elkholly, D. A., Inui, K., Padungtod, P., Newman, S. H., Fournié, G., & Pfeiffer, D. U. (2019). Optimising the detectability of H5N1 and H5N6 highly pathogenic avian influenza viruses in Vietnamese live-bird markets. *Scientific Reports*, *9*, 1031.

Wang, M., Di, B., Zhou, D. H., Zheng, B. J., Jing, H., Lin, Y. P., Liu, Y. F., Wu, X. W., Qin, P. Z., Wang, Y. L., Jian, L. Y., Li, X. Z., Xu, J. X., Lu, E. J., Li, T. G., & Xu, J. (2006). Food markets with live birds as source of avian influenza. *Emerging Infectious Diseases.*, *12*, 1773–1775.

Wang, X., Bailey, E. S., Qi, X., Yu, H., Bao, C., & Gray, G. C. (2020). Bioaerosol sampling at a live animal market in Kunshan, China: A noninvasive approach for detecting emergent viruses. *Open Forum Infectious Diseases.*, *7*, ofaa134.

Wu, Y., Lin, J., Yang, S., Xie, Y., Wang, M., Chen, X., Zhu, Y., Luo, L., & Shi, W. (2018). The molecular characteristics of avian influenza viruses (H9N2) derived from air samples in live poultry markets. *Infection, Genetics and Evolution.*, *60*, 191–196.

Wu, Y., Shi, W., Lin, J., Wang, M., Chen, X., Liu, K., Xie, Y., Luo, L., Anderson, B. D., Lednicky, J. A., Gray, G. C., Lu, J., & Wang, T. (2017). Aerosolized avian influenza a (H5N6) virus isolated from a live poultry market, China. *Journal of Infection.*, *74*, 89–91.

Zeng, X., Liu, M., Zhang, H., Wu, J., Zhao, X., Chen, W., Yang, L., He, F., Fan, G., Wang, D., Chen, H., & Sju, Y. (2017). Avian influenza H9N2 virus isolated from air samples in LPMs in Jiangxi, China. *Virology Journal.*, *14*, 136.

Zhou, J., Wu, J., Zeng, X., Huang, G., Zou, L., Song, Y., Gopinath, D., Zhang, X., Kang, M., Lin, J., Cowling, B. J., Lindsley, W. G., Ke, C., Peiris, J. S. M., & Yen, H. L. (2016). Isolation of H5N6, H7N9 and H9N2 avian influenza a viruses from air sampled at live poultry markets in China, 2014 and 2015. *Eurosurveillance*, *21*, 30331.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Horwood, P. F., Horm, S. V., Yann, S., Tok, S., Chan, M., Suttie, A., Y, P., Rith, S., Siegers, J. Y., San, S., Davun, H., Tum, S., Ly, S., Tarantola, A., Dussart, P., & Karlsson, E. A. (2023). Aerosol exposure of live bird market workers to viable influenza A/H5N1 and A/H9N2 viruses, Cambodia. *Zoonoses and Public Health*, *70*, 171–175. https://doi.org/10.1111/zph.13009

18632378, 2023, 2, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/zph.13009 by Institut Pasteur, Wiley Online Library on [16/05/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License