

One Health in the Dairy Industry: Employing a One Health Approach in Studying Cross-species Respiratory Diseases on Dairy Farms

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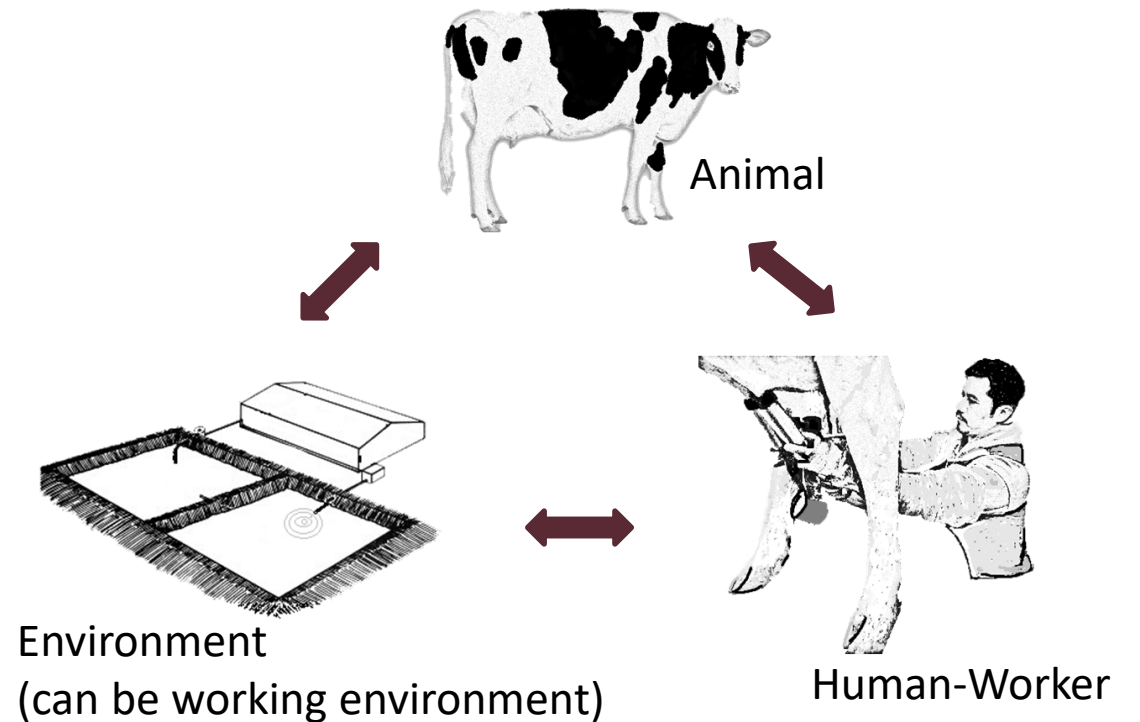
- Headquartered at Colorado State University
- One of 11 NIOSH-funded Centers for Agricultural Safety and Health
- International Dairy Research Consortium
 - Collaboration of international researchers interested in dairy (livestock) worker health and safety research and outreach
 - Open to all who are interested

One Health

- collaborative, multisectoral, and transdisciplinary approach
- working at the local, regional, national, and global levels
- with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment.

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One Health in Dairy



Why Take a One Health Approach
to Understand Respiratory Health
in Livestock and Humans?



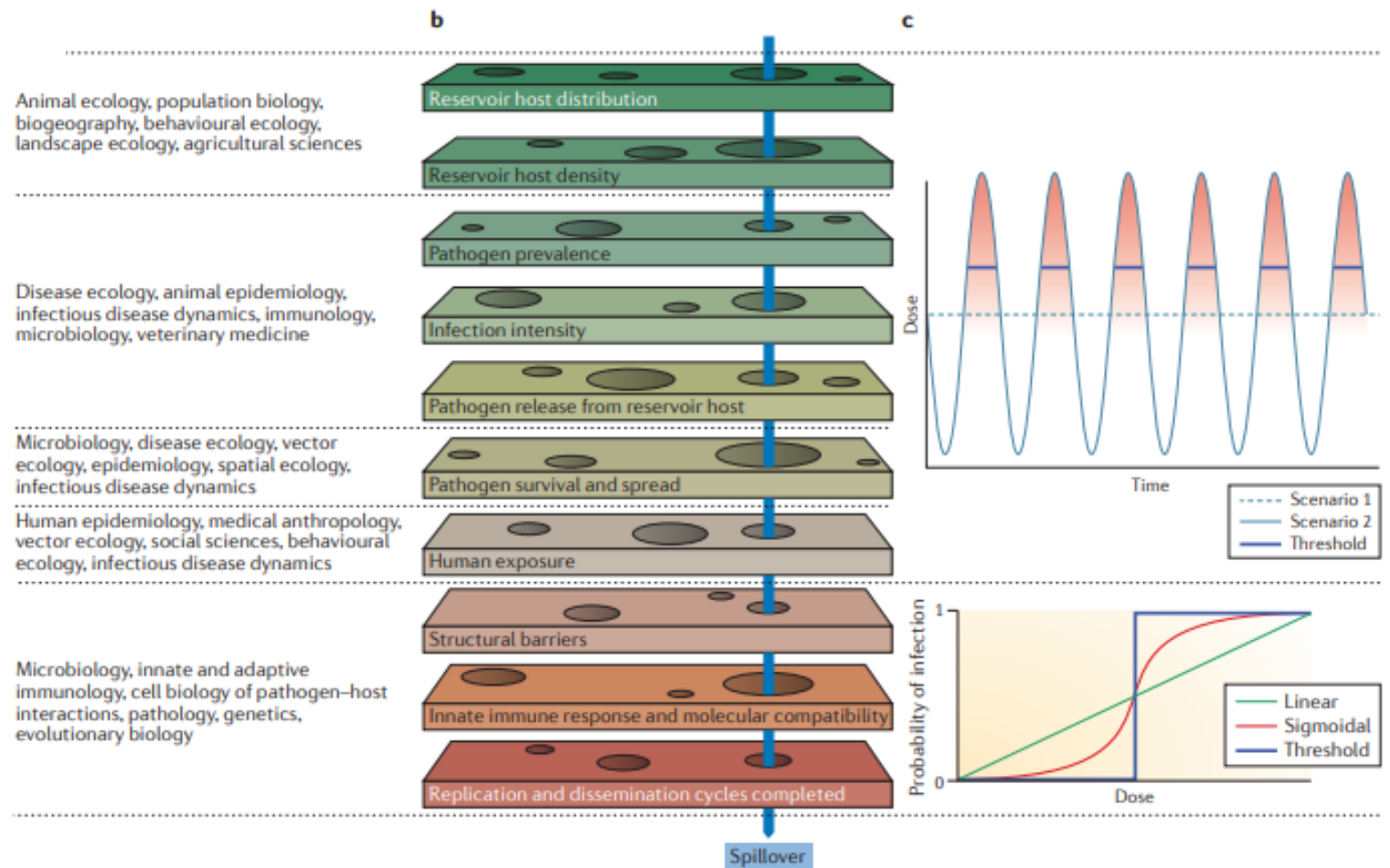
PERSPECTIVES

OPINION

Pathways to zoonotic spillover

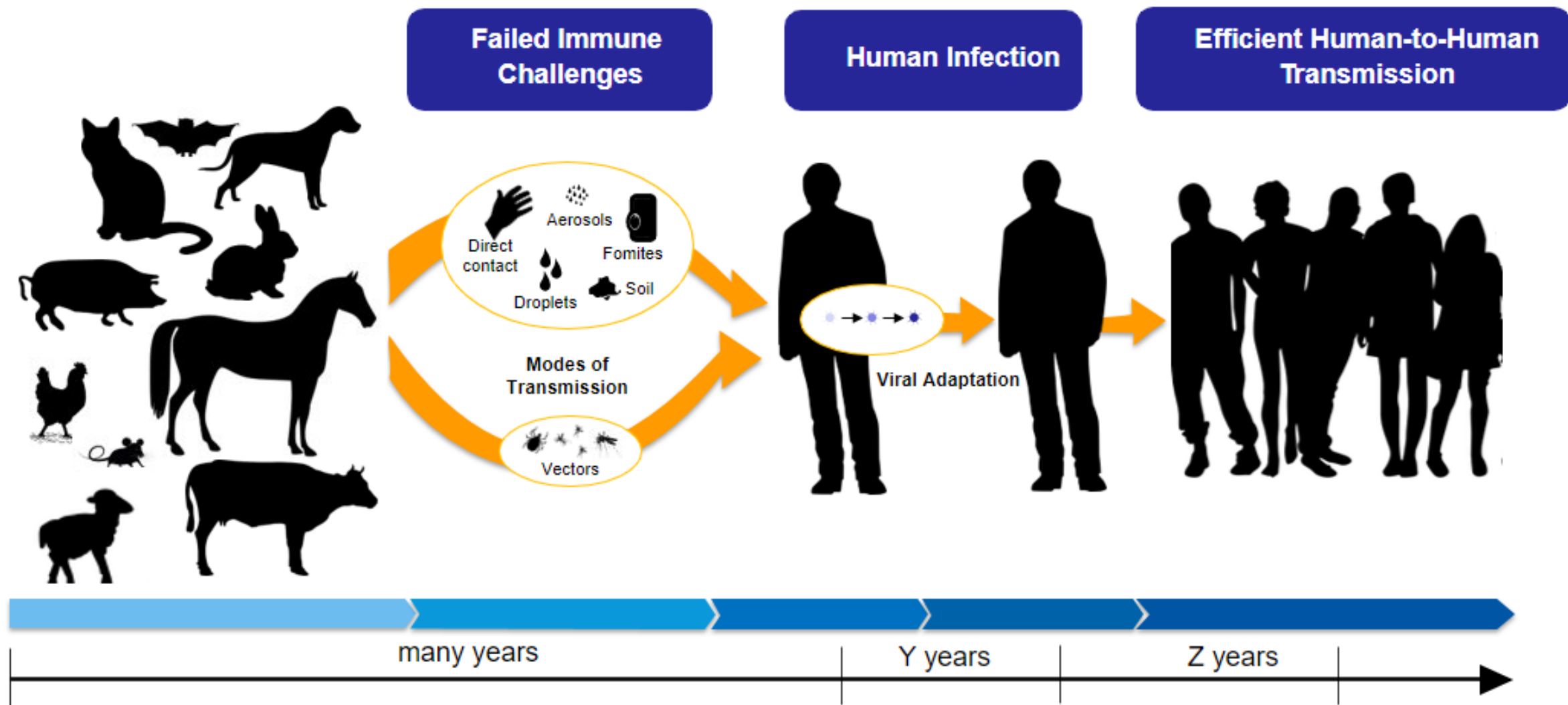
Raina K. Plowright, Colin R. Parrish, Hamish McCallum, Peter J. Hudson, Albert I. Ko, Andrea L. Graham and James O. Lloyd-Smith

There are many barriers that must be overcome for a pathogen to spillover into a new host and therefore is a rare event.



A Model for Zoonotic Pathogen Genesis

Viral evolutionary studies suggest this progression may take many years



ESSAY

How accurately can we assess zoonotic risk?

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1 Marie Bashir Institute for Infectious Diseases and Biosecurity, School of Life and Environmental Sciences and School of Medical Sciences, The University of Sydney, Sydney, Australia, **2** Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand, **3** Institute of Environmental Science and Research, Wellington, New Zealand

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Abstract

Identifying the animal reservoirs from which zoonotic viruses will likely emerge is central to understanding the determinants of disease emergence. Accordingly, there has been an increase in studies attempting zoonotic “risk assessment.” Herein, we demonstrate that the virological data on which these analyses are conducted are incomplete, biased, and rapidly changing with ongoing virus discovery. Together, these shortcomings suggest that attempts to assess zoonotic risk using available virological data are likely to be inaccurate and largely only identify those host taxa that have been studied most extensively. We suggest that virus surveillance at the human–animal interface may be more productive.

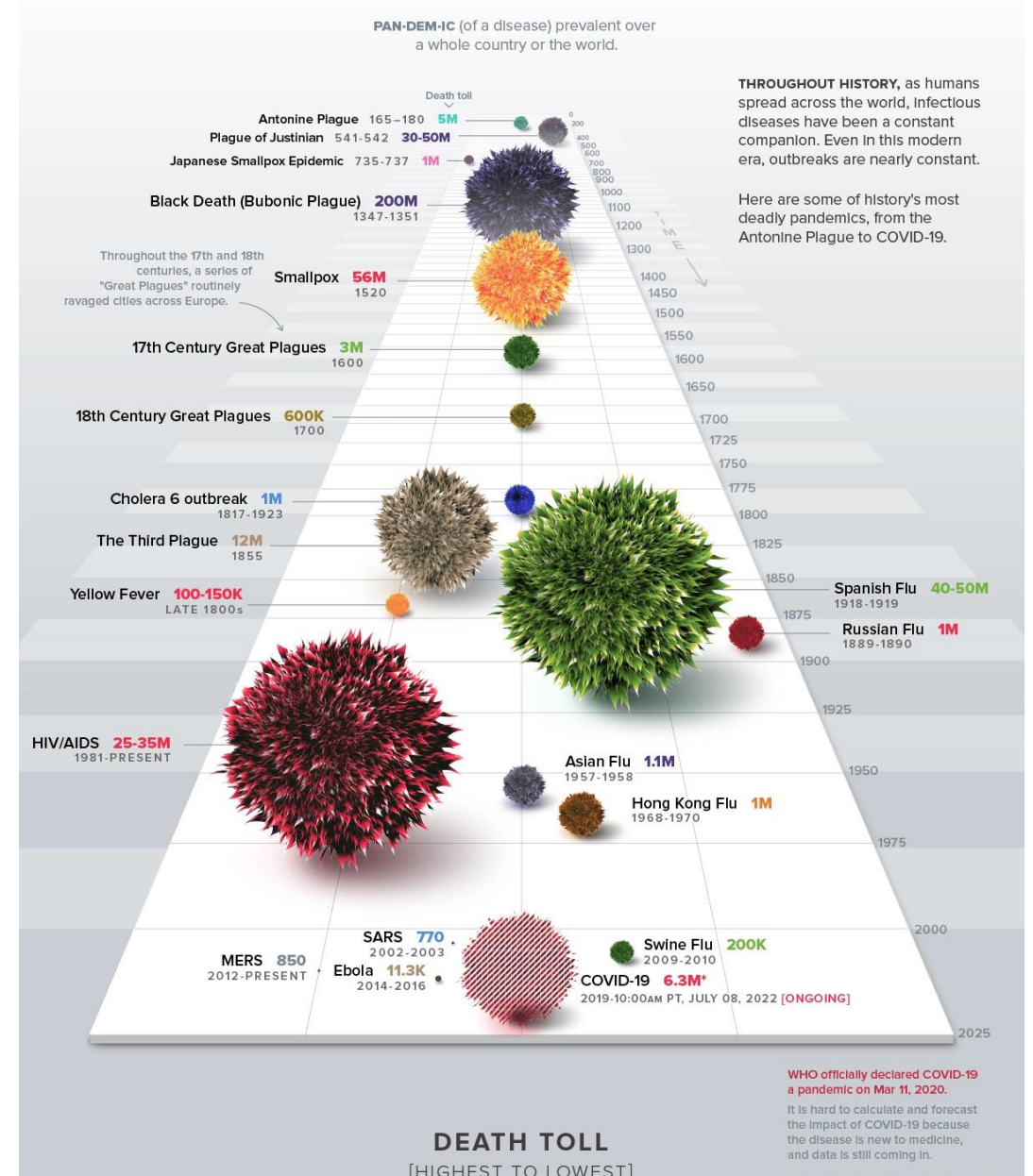
“Given the enormity of the virosphere, that RNA viruses evolve so quickly that repeat sampling will be regularly required to accurately track natural diversity, and that virome composition will likely vary across the geographic range occupied by an individual host species, **a more targeted, and arguably more productive, approach will be to focus sampling directly at the animal–human interface.**”



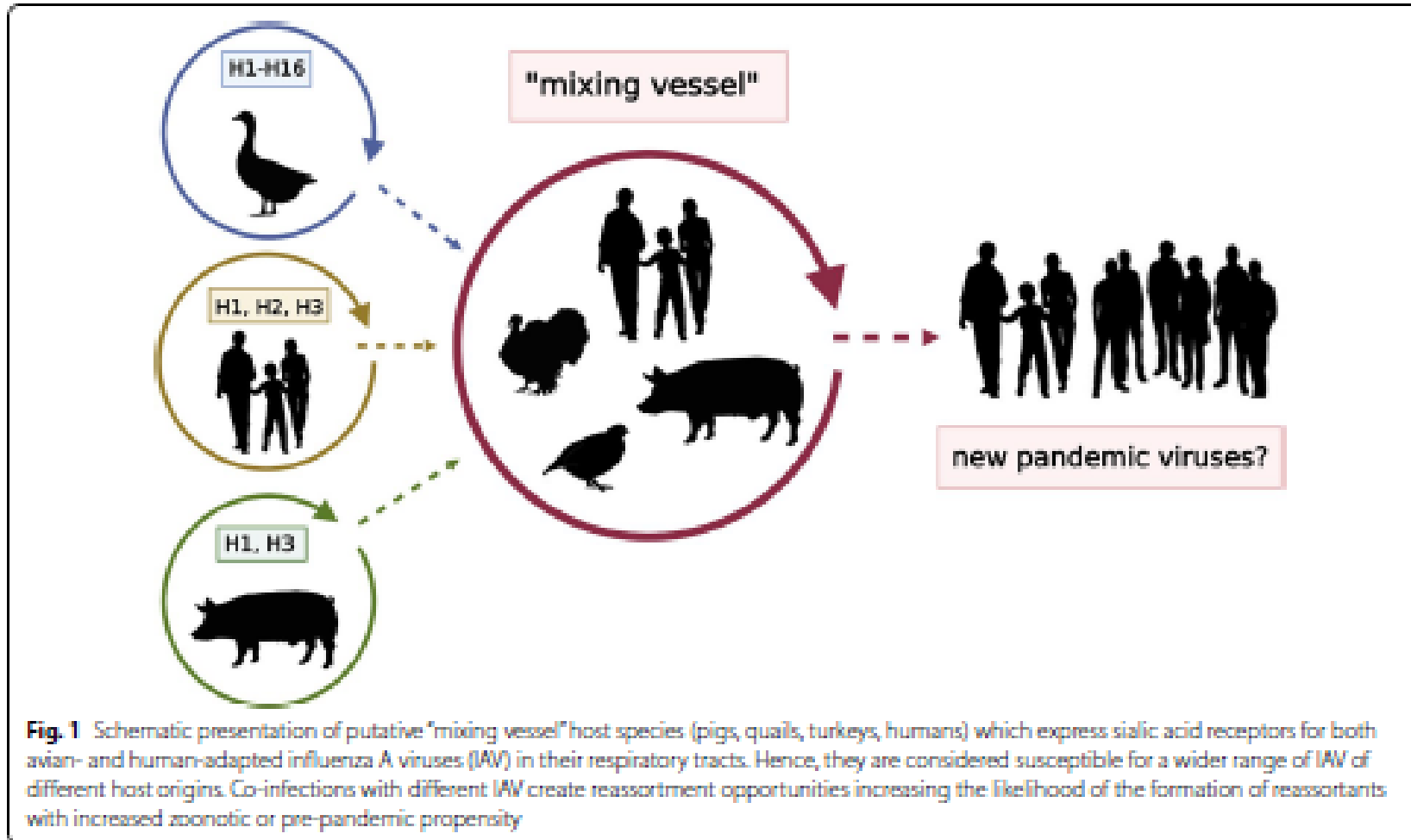
Why respiratory pathogens?

- Respiratory diseases are the most frequent cause of epidemics and pandemics
- Also have a high morbidity and mortality and are therefore the target of multiple interventions

HISTORY OF PANDEMICS



Animals as sources of zoonotic pathogens



Reservoir-Human Contact Rate / Zoonotic Pathogen Prevalence in Animal Reservoirs

International Journal of Infectious Diseases 88 (2019) 113–119



Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid



Review

Animal influenza virus infections in humans: A commentary

Laura K. Borkenhagen^a, Mo D. Salman^b, Mai-Juan Ma^c, Gregory C. Gray^{a,d,e,*}



Species barrier is lowest for influenza A transmission between pigs and humans

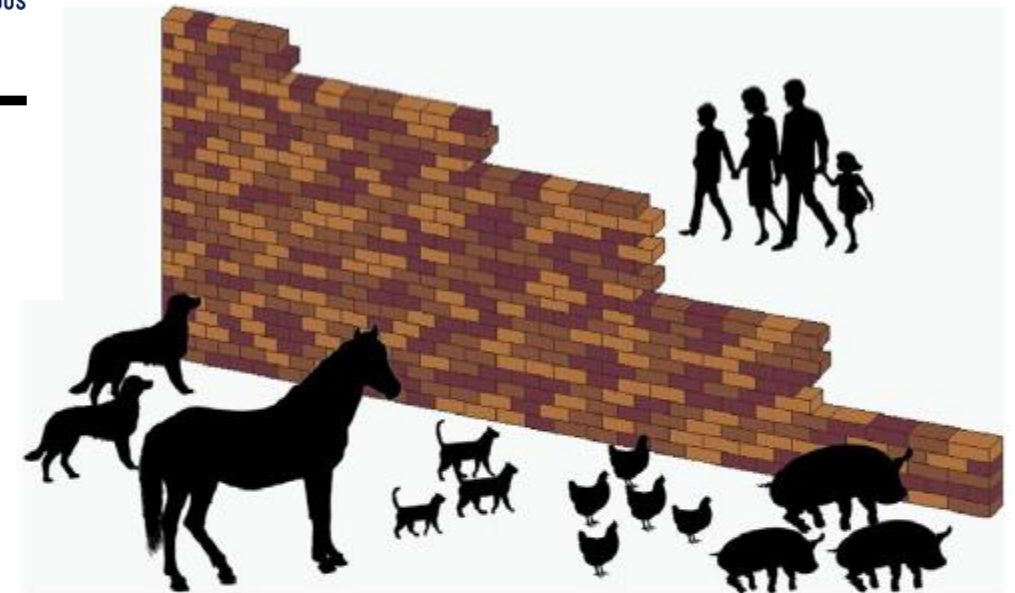
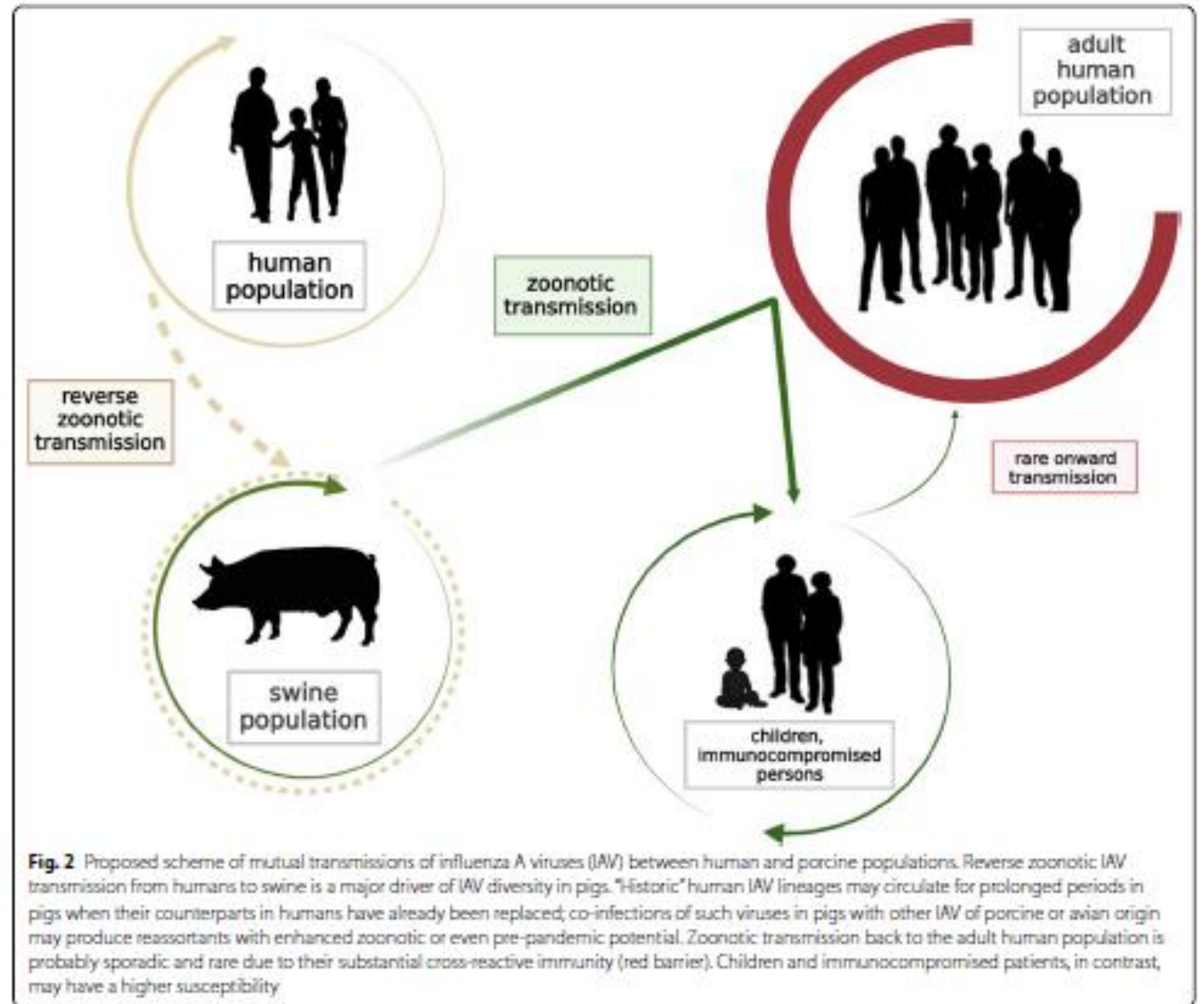


Figure 2. This is a qualitative, graphical depiction of the authors' assessment of the current risk of influenza A viruses crossing from various domestic animals to infect humans. The graphic is composite or gestalt of case reports, sero-epidemiological studies, archeo-epidemiological studies, environmental studies, and historical observations. The authors' intent with this graphic is to illustrate the high relative probability of continued movement of influenza A viruses between pigs and humans, contrasting that with the knowledge that swine viruses have been associated with at least four recent pandemics, yet worldwide we continue to have relatively sparse surveillance for novel influenza A virus detections among pigs.

Reverse Zoonoses



Reverse
zoonoses
from
farmworker
to animals



Received: 5 January 2022 | Revised: 18 February 2022 | Accepted: 2 April 2022

DOI: 10.1111/zph.12948

ORIGINAL ARTICLE

WILEY

Evidence of influenza A infection and risk of transmission between pigs and farmworkers

Gustavo Lopez-Moreno¹ | Peter Davies¹  | My Yang¹ | Marie R. Culhane¹ | Cesar A. Corzo¹ | Chong Li¹ | Aaron Rendahl² | Montserrat Torremorell¹ 


Found 1) evidence of swine-origin influenza A strains present in nasal passages of swine workers after work and 2) that some swine workers were IAV-RNA positive at farm entry and exit and therefore could introduce new strains that could impact pigs.

RESEARCH ARTICLE

Open Access



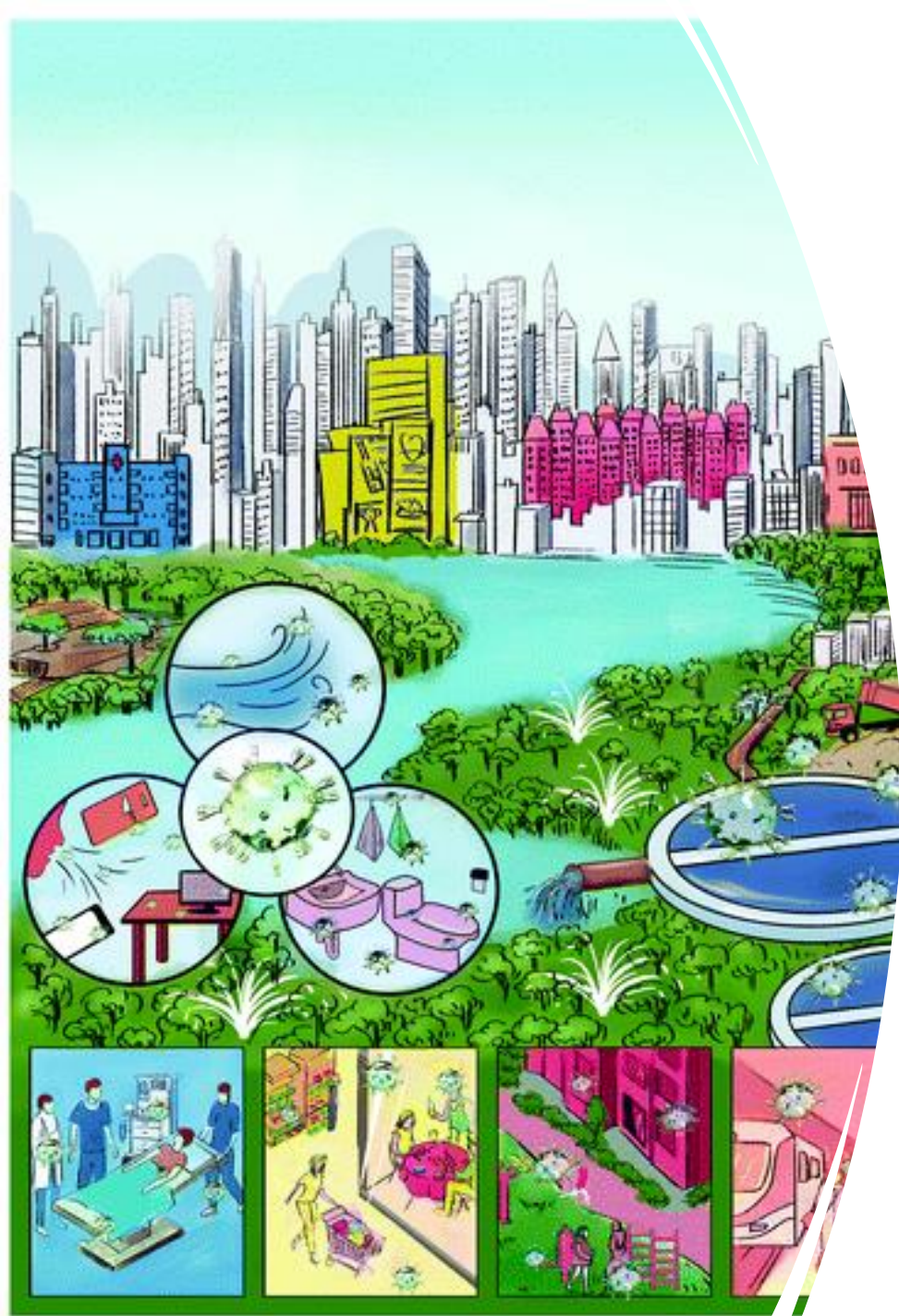
Temporary carriage of bovine coronavirus and bovine respiratory syncytial virus by fomites and human nasal mucosa after exposure to infected calves

Veslemøy Sunniva Oma^{1*} , Thea Klem¹, Madeleine Tråvén², Stefan Alenius², Britt Gjerset³, Mette Myrmet⁴ and Maria Stokstad¹

For BCoV, infective virus was detected after 24 hrs on fomites (clothes, boots, wrist-watches, etc.)...Viral BCoV and BRSV RNA was also detected in human nasal mucosa after exposure to animals, but no infective virus was found.

Viral Surveillance in the Environment

- Non-invasive surveillance
 - Aerosols
 - Wastewater
 - Fomites



CORONAVIRUS

Airborne transmission of respiratory viruses

Chia C. Wang^{1,2*}, Kimberly A. Prather^{3*}, Josué Sznitman⁴, Jose L. Jimenez⁵, Seema S. Lakdawala⁶, Zeynep Tufekci⁷, Linsey C. Marr⁸

Table 1. Airborne transmission of respiratory viruses. Representative evidence of airborne transmission for various respiratory viruses and their basic reproduction number. Cells with dashes indicate not applicable.

Virus name	Scope of studies and/or approaches						Basic reproduction number (R_0)	
	Air sampling and PCR	Air sampling and cell culture	Animal models	Laboratory or clinical studies	Epidemiological analysis	Simulation and modeling		Size-resolved information
SARS-CoV	(31)	(31)	–	(30)	(30)	(30)	–	2.0–3.0 (197)
MERS-CoV	(32)	(32, 103)	(103, 198)	(32)	–	–	–	0.50–0.92 (197)
SARS-CoV-2	(41–44)	(34, 35, 40)	(33, 37, 199)	(34, 45, 107)	(36, 64, 71, 72, 186)	(36, 50)	(34, 41, 43)	1.4–8.9 (57, 58)
Influenza virus	(22, 23, 98, 102, 106)	(23, 98, 101)	(24, 137, 200, 201)	(24, 138, 202, 203)	(20)	(20, 114, 204)	(23, 105, 106)	1.0–21 (205)
Rhinovirus	(9, 27)	(26, 28)	–	(26–28)	–	(27)	(9)	1.2–2.7 (205)
Measles virus	(16)	(16)	–	–	(17)	(17)	(16)	12–18 (206)
Respiratory syncytial virus (RSV)	(102)	(25)	–	(25)	–	–	(25)	0.9–21.9 (205)

Bioaerosol Sampling to Detect Avian Influenza Virus in Hanoi's Largest Live Poultry Market

Vuong N. Bui,¹ Tham T. Nguyen,² Hung Nguyen-Viet,^{3,4} Anh N. Bui,¹ Katie A. McCallion,⁵ Hu Suk Lee,³ Son T. Than,¹ Kristen K. Coleman,² and Gregory C. Gray^{2,6,7}

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Background. Newly emergent and virulent strains of H7N9 avian influenza virus are rapidly spreading in China and threaten to invade Vietnam. We sought to introduce aerosol sampling for avian influenza viruses in Vietnam.

Methods. During October 2017, National Institute for Occupational Safety and Health 2-stage aerosol samplers were assembled on a tripod and run for 4 hours. Concomitantly, up to 20 oropharyngeal (OP) swab samples were collected from chickens and ducks distanced at 0.2–1.5 m from each sampler.

Results. The 3 weeks of sampling yielded 30 aerosol samples that were 90% positive for influenza A, by quantitative reverse-transcription polymerase chain reaction, and 116 OP swab sample pools (5 samples per pool) that were 47% positive. Egg cultures yielded 1 influenza A virus (not H5 or H7) from aerosol and 25 influenza A viruses from OP swab sample pools (5 were H5 positive). The association between positive sample types (over time and position) was strong, with 91.7% of positive OP pooled swab samples confirmed by positive aerosol samples and 81% of influenza A positive aerosol samples confirmed by positive OP swab samples.

Conclusions. We posit that aerosol sampling might be used for early warning screening of poultry markets for novel influenza virus detection, such as H7N9. Markets with positive aerosol samples might be followed up with more focused individual bird or cage swabbing, and back-tracing could be performed later to locate specific farms harboring novel virus. Culling birds in such farms could reduce highly pathogenic avian influenza virus spread among poultry and humans.

Keywords. avian influenza; influenza A virus; Vietnam; poultry; epidemiology.



Dr. Vuong N. Bui



Dr. Hung Nguyen-Viet



Dr. Teck-Hock Toh



Gregory C. Gray



Xinye Wang

Bioaerosol Sampling at a Live Animal Market in Kunshan, China: A Noninvasive Approach for Detecting Emergent Viruses

Xinye Wang,¹ Emily S. Bailey,^{2,3,4} Xian Qi,⁵ Huiyan Yu,⁶ Changjun Bao,⁵ and Gregory C. Gray^{1,2,3,4,5}

¹Global Health Research Center, Duke Kunshan University, Kunshan, China, ²Division of Infectious Diseases, School of Medicine, Duke University, Durham, North Carolina, USA, ³Global Health Institute, Duke University, Durham, North Carolina, USA, ⁴Julia Jones Matthews Department of Public Health, Texas Tech University Health Sciences Center, Abilene, Texas, USA, ⁵Department of Acute Infectious Disease, Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China, ⁶Nicholas School, Duke University, Durham, North Carolina, USA, and ⁷Emerging Infectious Diseases Program, Duke-NUS Medical School, Singapore

Keywords. avian influenza viruses; infectious aerosol; one health; phylogenetic analysis; poultry market.

Chinese LBMs often have multiple species of live poultry for sale, including chickens, ducks, quails, geese, and pigeons. These different species of live poultry are often mixed in cages and have beak-to-beak contact, which significantly increases the risk of AIV transmission across species. Additionally, poultry are often purchased and butchered on site, with little concern for potential human exposure or biosecurity. Such LBM conditions may pose a threat to public health.

In this pilot study, we sought to examine molecular evidence for influenza A, B, C, and D viruses in a single LBM located in Kunshan, China.

METHODS

Ethics Statement and Study Location

Bioaerosol samples were obtained from a small LBM (6 small open stalls and 1 poultry slaughter room) in Kunshan City,



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Noninvasive Surveillance for Emerging Pathogens on Farms

Received: 28 August 2019 | Revised: 19 May 2020 | Accepted: 7 June 2020
DOI: 10.1111/tbed.13683



ORIGINAL ARTICLE

Transboundary and Emerging Diseases | WILEY

Environmental bioaerosol surveillance as an early warning system for pathogen detection in North Carolina swine farms: A pilot study

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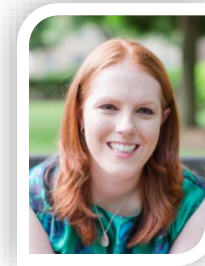
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⁷Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, Singapore

Abstract

Disease outbreaks can readily threaten swine production operations sometimes resulting in large economic losses. Pathogen surveillance in swine farms can be an effective approach for the early identification of new disease threats and the mitigation of transmission before broad dissemination among a herd occurs. Non-invasive environmental bioaerosol sampling could be an effective and affordable approach for conducting routine surveillance in farms, providing an additional tool for farmers to protect their animals and themselves from new disease threats. In this pilot study, we implemented a non-invasive, prospective bioaerosol sampling strategy in a swine farm located in the United States to detect economically important swine pathogens. Farm personnel collected air samples from two swine barns for 23 weeks between July and December 2017. Samples were then tested within 24 hr of collection by molecular techniques for a number of economically important swine pathogens. Of the 86 bioaerosol samples collected, 4 (4.7%) were positive for influenza A, 1 (1.2%)



SCIENTIFIC REPORTS

nature research



A feasibility study of conducting surveillance for swine pathogens in slurry from North Carolina swine farms

Emily S. Bailey^{1,2,3,6,7}, Laura K. Borkenhagen^{1,2}, Jessica Y. Choi^{1,2}, Annette E. Greer⁴, Marie R. Culhane⁵ & Gregory C. Gray^{1,2,6,7}

Despite close contact between humans and animals on large scale farms, little to no infectious disease research is conducted at this interface. Our goal in this preliminary study was to explore if we could detect swine pathogens using a non-invasive, indirect approach through the study of swine slurry. From April to November 2018, 105 swine slurry samples were collected by farm personnel from waste pits at two sites on a swine farm in North Carolina. These samples were tested for DNA and RNA viruses using a real-time PCR and RT-PCR. Statistical analyses were performed to measure association between virus positive outcomes and potential predictors such as date of sample collection, weight of pigs, number of pigs in barn, temperature, and weather conditions. Overall, 86% of the samples had evidence of at least one of the targeted viruses. Ultimately, this study demonstrated the utility of conducting noninvasive surveillance for swine pathogens through the study of swine slurry. Such swine slurry surveillance may supplant the need to handle, restrain, and collect specimens directly from pigs thus providing an approach to emerging pathogen detection that appeals to the swine industry.

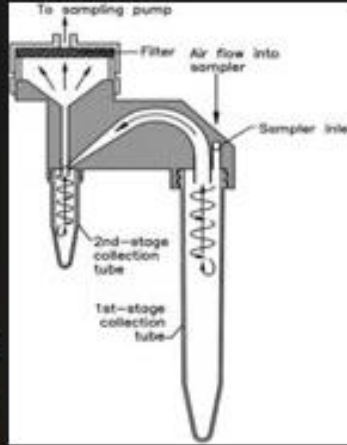
Bioaerosol Samplers



SKC BioSampler



NIOSH 2-stage sampler
BC 251 sampler



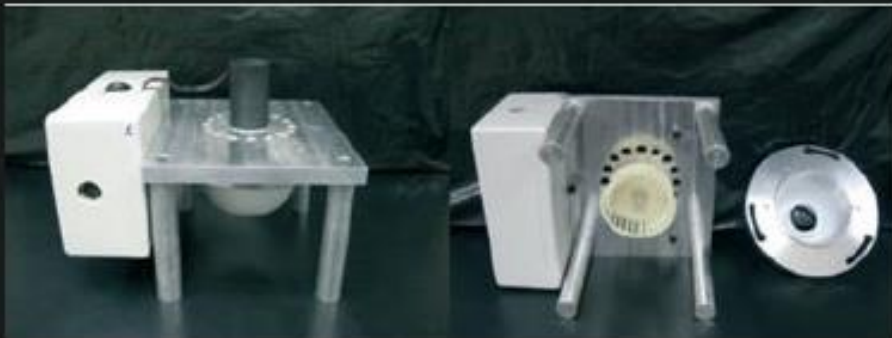
SIOUTAS PERSONAL CASCADE IMPACTOR

Four impaction stages
and after-filter:

- 2.5-10 μm
- 1.0-2.5 μm
- 0.5-1.0 μm
- 0.25-0.5 μm
- <0.25 μm



SKC Cat. No. 225-370



Midwest Micro Tek air sampling kits



SKC pump and personal sampler w/filter



Midget Impingers

The catch-all approach

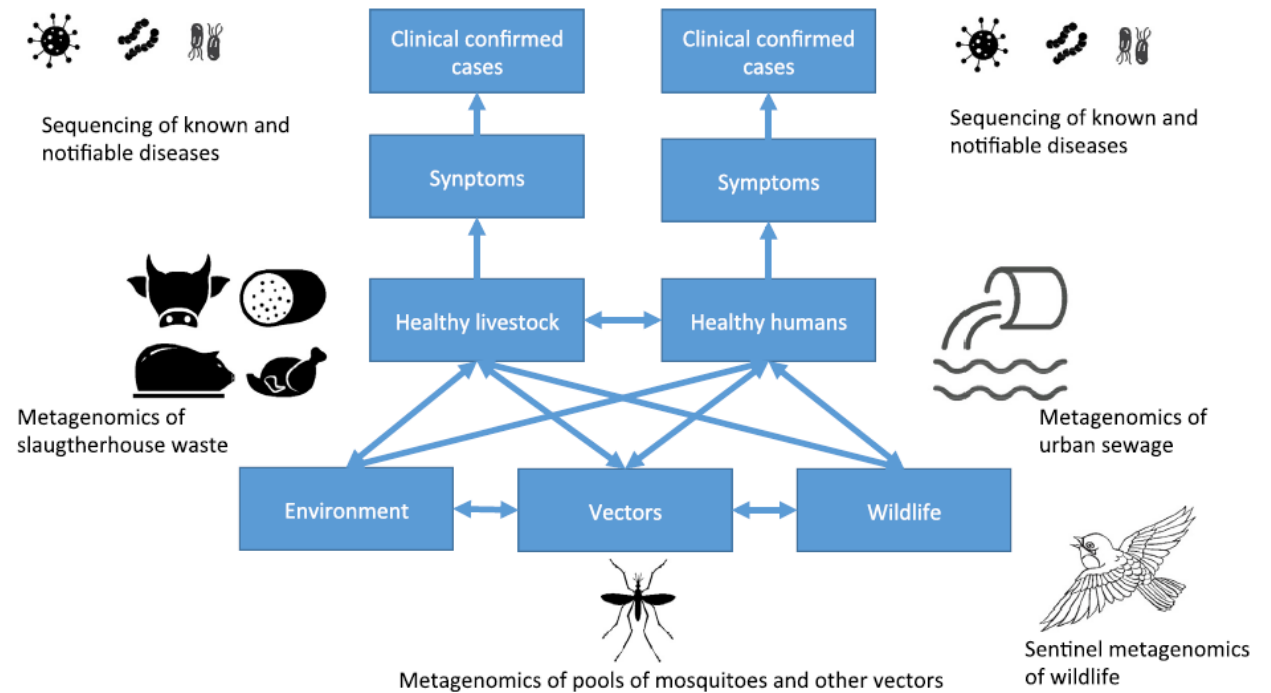
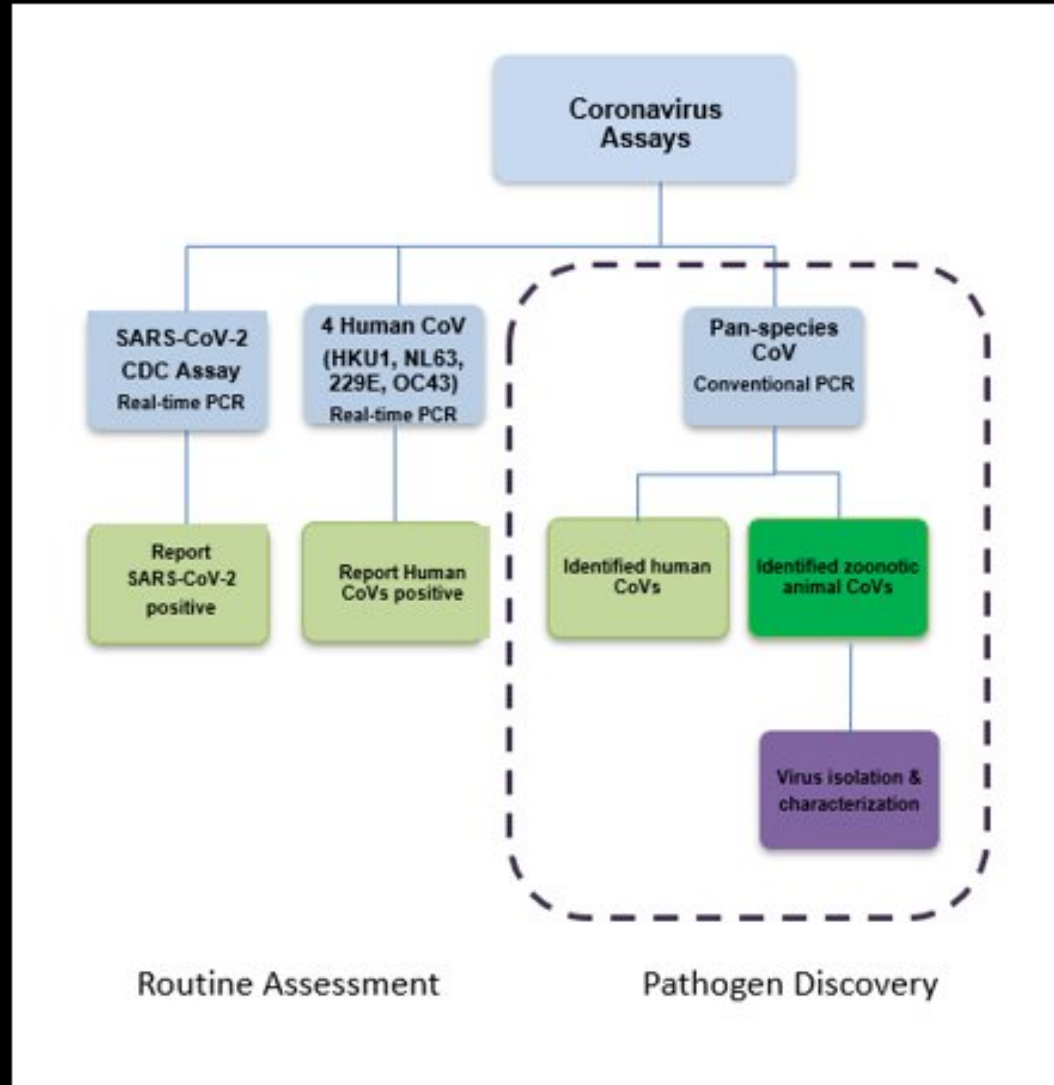


Fig. 1. Examples for catch-all surveillance, combining disease surveillance in animals and humans targeting known diseases (top row), with catch-all metagenomic surveillance capturing circulation on other pathogens in livestock or humans (in this example depicted as wastewater metagenomics in slaughterhouses and urban sewage, respectively). Additional metagenomic sequencing could include other environmental samples, samples from wild-life and from vectors.

Aarestrup et al., 2021. Pandemics – One Health preparedness for the next. The Lancet Regional Health – Europe 9. 100210.

Six Key Viral Families for the Zoonotic Viruses

Adenoviridae
Coronaviridae
Orthomyxoviridae
Picornaviridae
Paramyxoviridae
Pneumoviridae



Opinion
Mitigating Future Respiratory Virus Pandemics: New Threats and Approaches to Consider

Gregory C. Gray ^{1,2,3,4,*}, Emily R. Robie ^{1,2}, Caleb J. Studstill ^{1,2} and Charles L. Nunn ^{2,5}

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Abstract: Despite many recent efforts to predict and control emerging infectious disease threats to humans, we failed to anticipate the zoonotic viruses which led to pandemics in 2009 and 2020. The morbidity, mortality, and economic costs of these pandemics have been staggering. We desperately need a more targeted, cost-efficient, and sustainable strategy to detect and mitigate future zoonotic respiratory virus threats. Evidence suggests that the transition from an animal virus to a human pathogen is incremental and requires a considerable number of spillover events and considerable time before a pandemic variant emerges. This evolutionary view argues for the refocusing of public health resources on novel respiratory virus surveillance at human–animal interfaces in geographical hotspots for emerging infectious diseases. Where human–animal interface surveillance is not possible, a secondary high-yield, cost-efficient strategy is to conduct novel respiratory virus surveillance among pneumonia patients in these same hotspots. When novel pathogens are discovered, they must be quickly assessed for their human risk and, if indicated, mitigation strategies initiated. In this review, we discuss the most common respiratory virus threats, current efforts at early emerging pathogen detection, and propose and defend new molecular pathogen discovery strategies with the goal of preempting future pandemics.

Keywords: pathogen discovery; molecular detection; respiratory viruses; emerging viruses



Citation: Gray, G.C.; Robie, E.R.; Studstill, C.J.; Nunn, C.L. Mitigating Future Respiratory Virus Pandemics: New Threats and Approaches to Consider. *Viruses* **2021**, *13*, 637. <https://doi.org/10.3390/v13040637>

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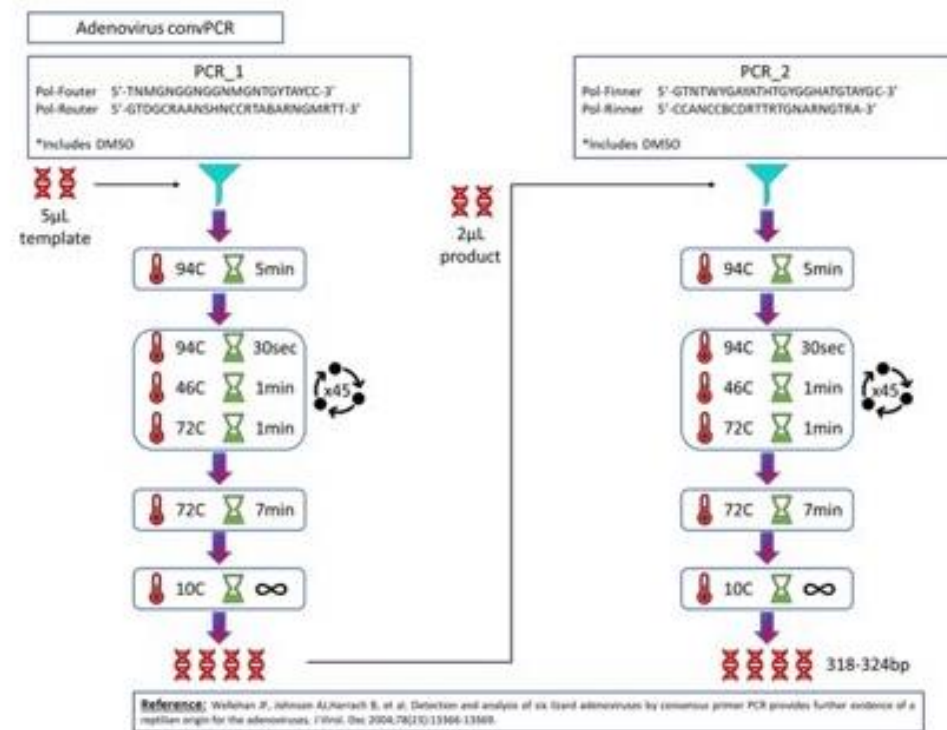
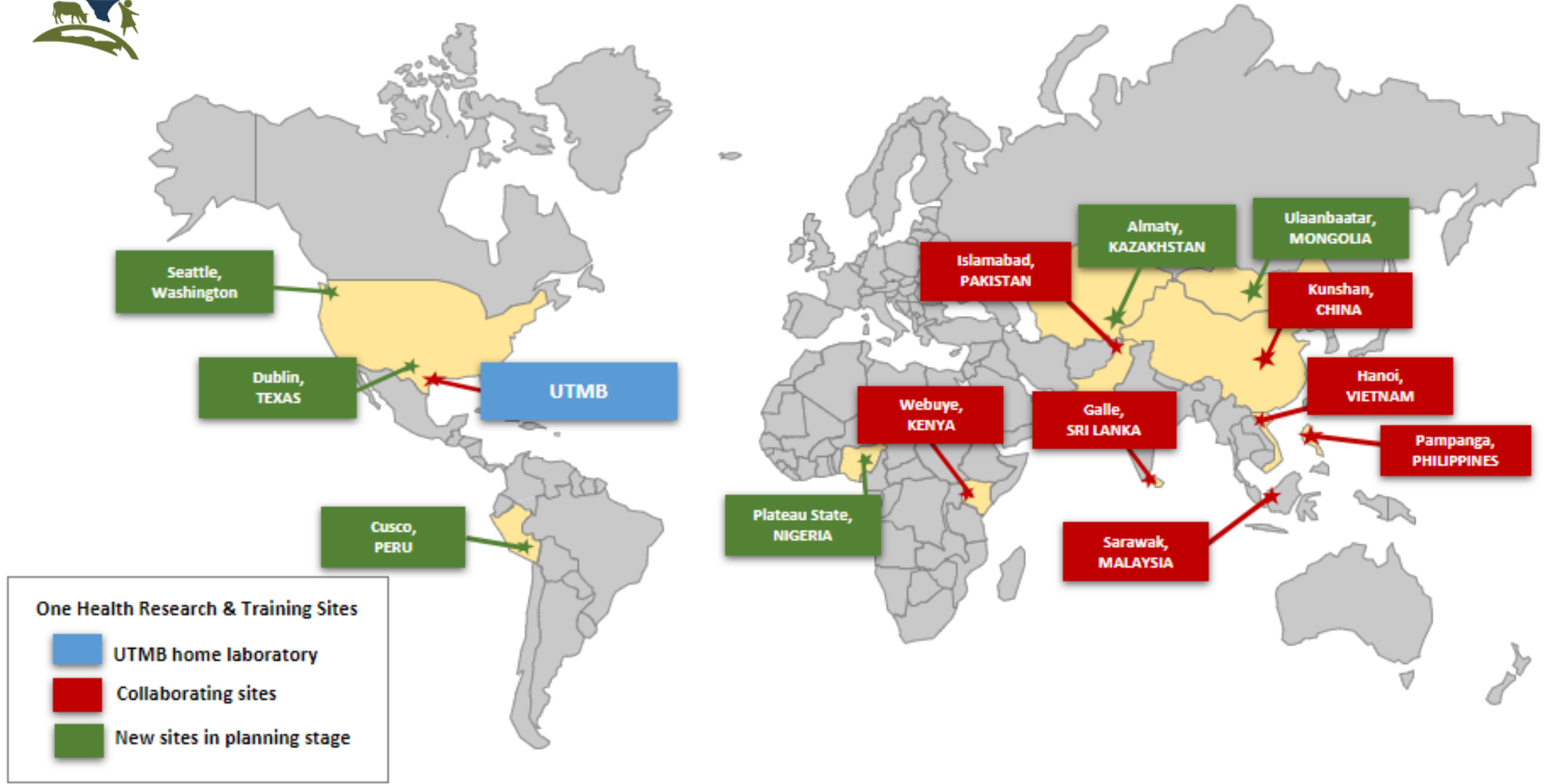



Figure S9. Detection of animal adenoviruses using conventional PCR (convPCR) [7]. The PCR 1 and PCR 2 primers recognizing adenovirus are listed. This assay utilizes the Platinum Taq DNA Polymerase kit (Invitrogen). For PCR 1, 0.5µL forward primer (25µM), 0.5µL reverse primer (25µM), 0.5µL dNTP (10mM), 0.24µL 100% dimethyl sulfoxide (DMSO), and 5µL extracted DNA are added to the PCR mix. The total volume of the reaction is 25µL. This is repeated for PCR 2 using the PCR 2 primers and 2µL of PCR 1 product.



Novel Zoonotic Respiratory Virus Detection Studies





Influenza D Virus

IDV Detections in Humans

Journal of Clinical Virology 81 (2016) 31–33



Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv



Short communication

Serologic evidence of exposure to influenza D virus among persons with occupational contact with cattle



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ARTICLE INFO

Article history:

Received 8 January 2016

Received in revised form 23 May 2016

Accepted 29 May 2016

Keywords:

Influenza

Influenza D virus

Zoonoses

Occupational health

ABSTRACT

Background: Influenza D virus (IDV), a novel influenza virus with proposed classification: family *Orthomyxoviridae*, genus *Influenzavirus D*, species *Influenza D virus*, has been associated with influenza-like illness in cattle and swine. More recently, anti-IDV antibodies have also been detected in small ruminants. A seroprevalence of approximately 1.3% has been estimated for the general human population.

Objectives: To gain insights on the zoonotic potential of IDV to human adults with occupational exposure to cattle in north central Florida.

Study: A cross-sectional serological study was performed on human serum samples from 35 cattle-exposed and 11 non-cattle-exposed adults to screen for IDV antibodies using hemagglutination inhibition (HI) and microneutralization (MN) assays.

Results: A seroprevalence of 91% was detected via HI assay, and 97% by MN assay among individuals working with cattle in Florida. Among non-cattle-exposed individuals, seropositivity determined via MN assay (only) was lower (18%).

Conclusions: IDV poses a zoonotic risk to cattle-exposed workers, based on detection of high seroprevalence (94–97%). Whereas it is still unknown whether IDV causes disease in humans, our studies indicate that the virus may be an emerging pathogen among cattle-workers.

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Detection of antibodies against influenza D virus in swine veterinarians in Italy in 2004

Claudia Maria Trombetta¹ | Emanuele Montomoli^{1,2,3} | Ilaria Di Bartolo⁴ | Fabio Ostanello⁵ | Chiara Chiapponi⁶ | Serena Marchi¹



Article

Influenza D Virus: Serological Evidence in the Italian Population from 2005 to 2017

Claudia M. Trombetta^{1,*}, Serena Marchi^{1,+}, Ilaria Manini^{1,+}, Otfried Kistner², Feng Li³, Pietro Piu², Alessandro Manenti⁴, Fabrizio Biuso⁴, Chithra Sreenivasan³, Julian Druce⁵ and Emanuele Montomoli^{1,2,4}

RESEARCH ARTICLE

Surveillance for respiratory and diarrheal pathogens at the human-pig interface in Sarawak, Malaysia

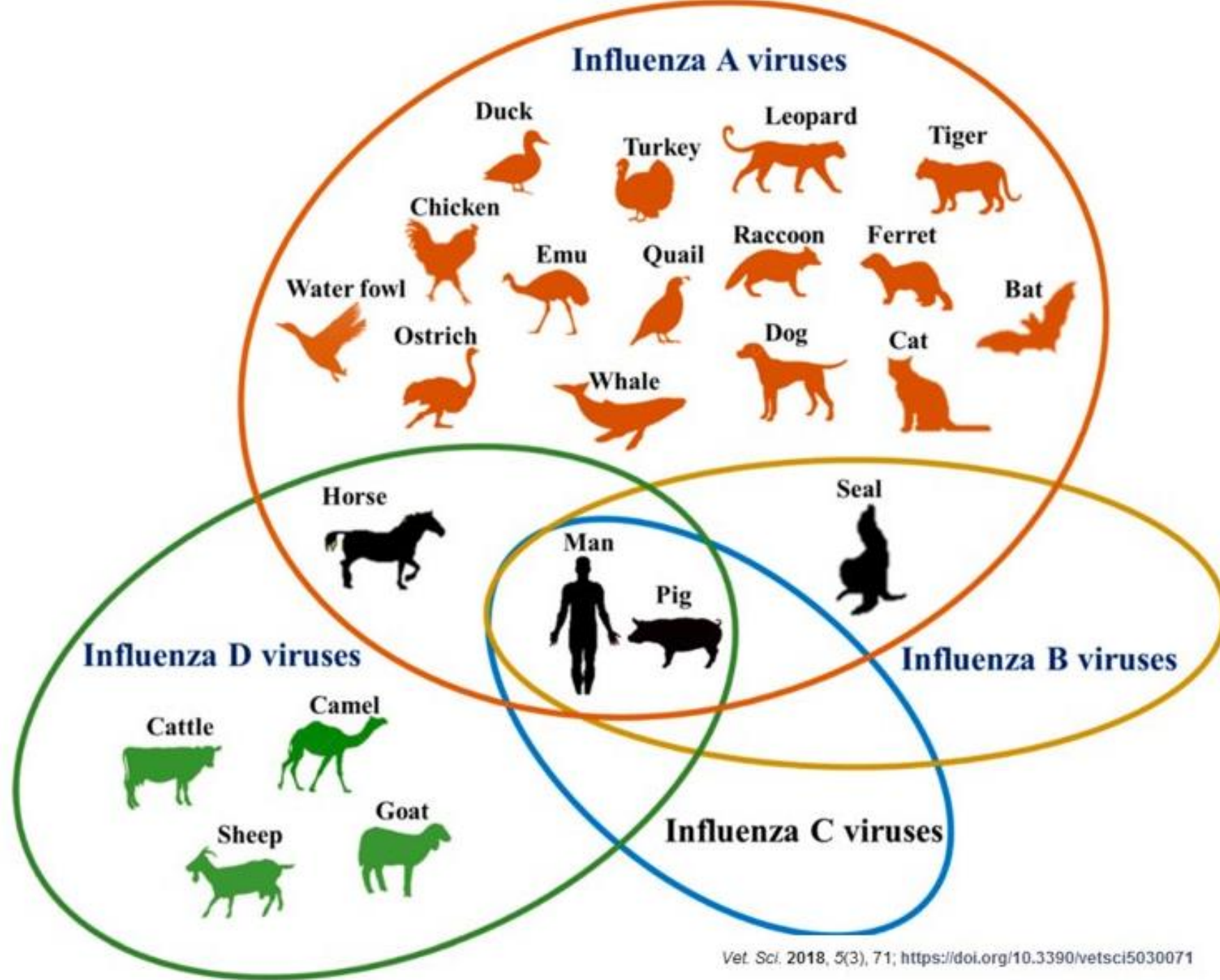
Laura K. Borkenhagen^{1,2*}, Kerry A. Mallinson^{1,c}, Rick W. Tsao^{1,c}, Siaw-Jing Ha^{3,4}, Wei-Honn Lim⁵, Teck-Hock Toh^{3,4,5}, Benjamin D. Anderson², Jane K. Fieldhouse^{1,2}, Sarah E. Philo^{1,2}, Kuek-Sen Chong^{3,6}, William G. Lindsley⁷, Alejandro Ramirez⁸, James F. Lowe⁹, Kristen K. Coleman¹⁰, Gregory C. Gray^{1,2,10}



BOVINE RESPIRATORY DISEASE (BRD)

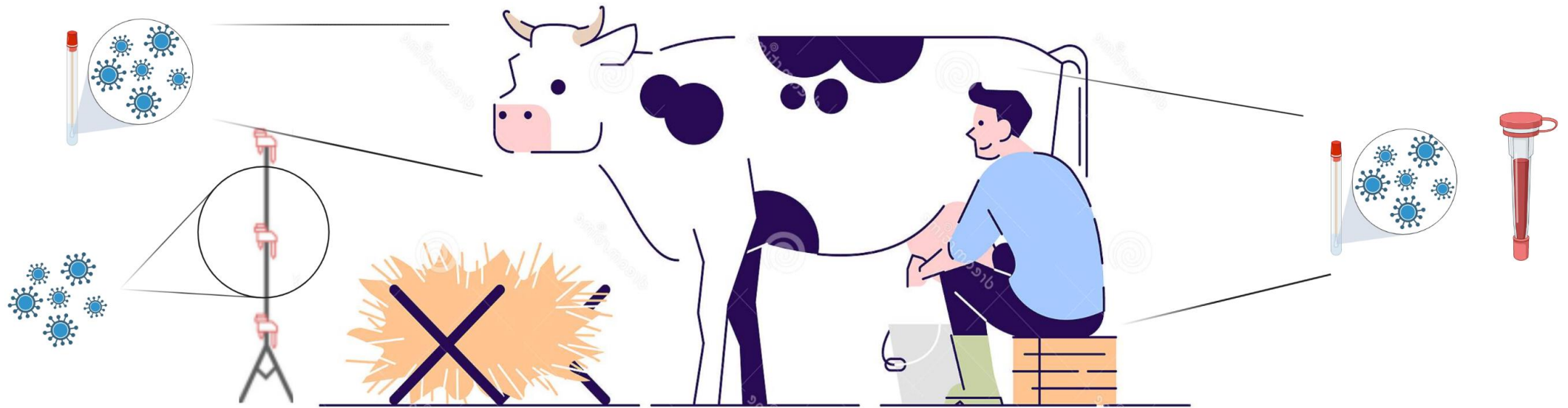
**BRD IS THE MOST DEVASTATING DISEASE OF
THE US CATTLE POPULATION.**

- Affects lower and upper respiratory tract of cattle, primarily young cattle
- Associated with various viruses and bacteria
- Estimated over \$3 Billion spent on prevention, treatment and losses annually



One Health Study Design

We work with farm owners to collect and study samples from farm workers, livestock, and the farm environment to better understand the epidemiology and ecology of respiratory pathogens.



Texas Food Security Novel Respiratory Virus Threat Project (CDC)

- In longitudinal One Health study we will prospectively follow two cohorts of **animal workers** working on farms in **Texas**.
- The workers' serial nasopharyngeal swabs the animals' oral secretions and farm bioaerosol samples will be studied with molecular assays for ***Adenoviridae*, *Coronaviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Picornaviridae* (chiefly enteroviruses), and *Pneumoviridae***.
- Next generation sequencing will also be employed on a subset of samples.

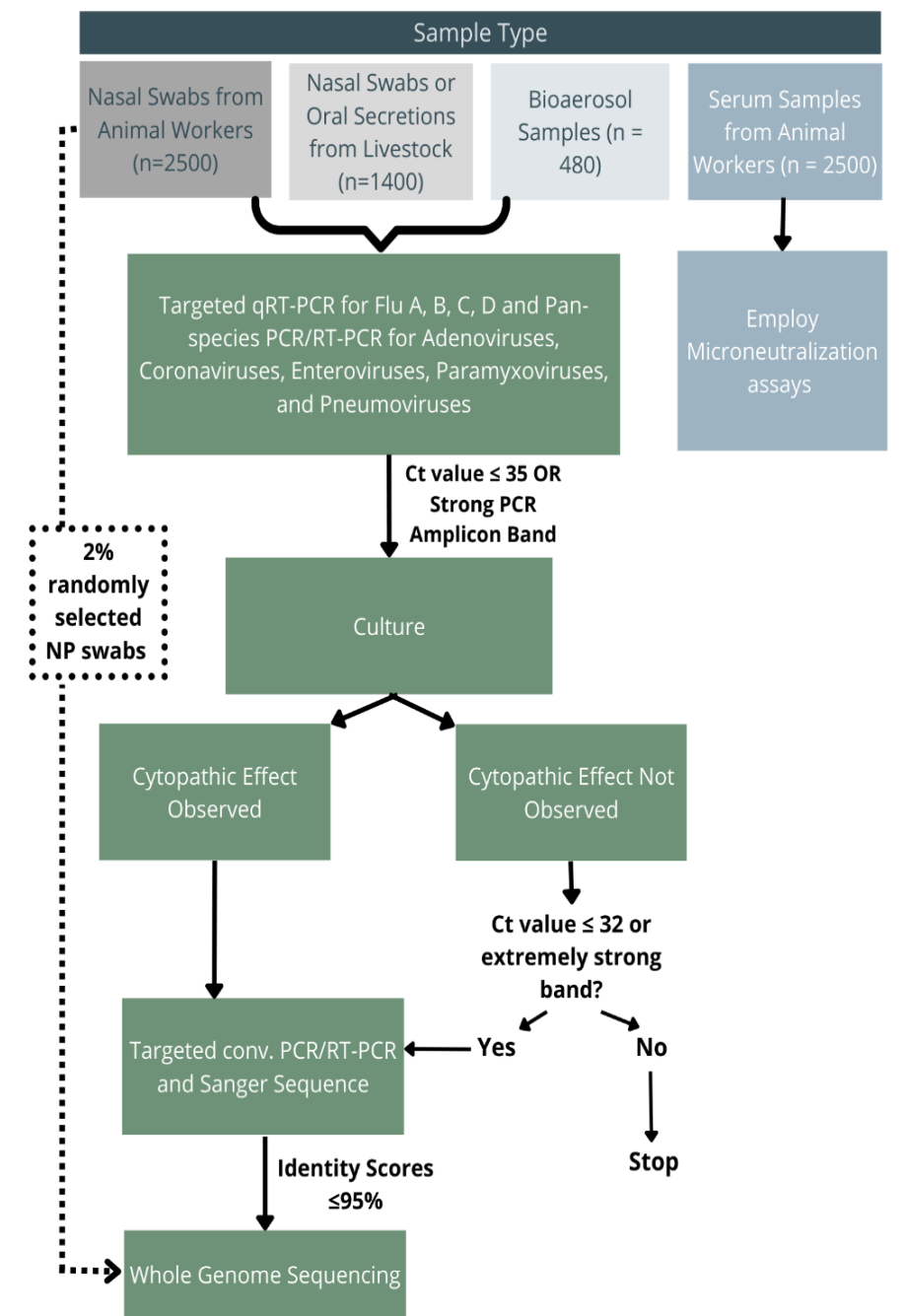
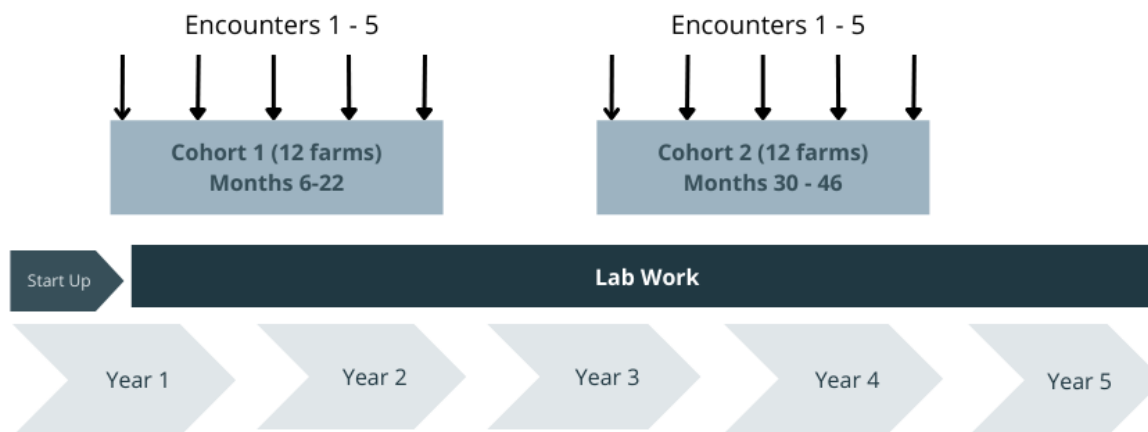


Fig. 2 Diagram illustrating planned laboratory analyses.

Rapid Detection of Incursions of SARS-CoV-2 and Novel Coronaviruses on Texas's Meat and Dairy Farms (USDA NIFA)

Using a One-Health oriented study design, we will use molecular and next generation sequencing techniques to surveil for SARS-CoV-2 and other coronaviruses in livestock farms (pigs, cattle, poultry) in Texas.

Should we find evidence of spillover into the human population we will employ serological analyses of workers' sera to assess the potential for spillover.

Lastly, in collaboration with GeneCapture, Inc., we will assess the utility of a new, farm-deployable, pan-species coronavirus diagnostic assay for use as a rapid diagnostic test on farms.



GeneCapture



Direct RNA hybridization and optical sensing



Reverse genetics studies of novel coronaviruses with UTMB's Vineet D. Menachery



Looking for
International
Collaborators to
Join the Effort!





Questions?

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