Transmission of influenza viruses

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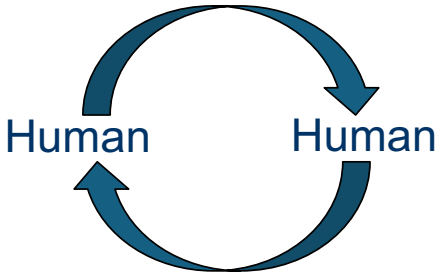
Croucher Summer Courses “Emerging Viral Infections”, 14-18 July 2025

Learning objectives

1. Modes of transmission and why it is important to learn about that?
2. How do influenza viruses transmit within and between species?
3. Can we develop effective countermeasures to prevent influenza transmission?

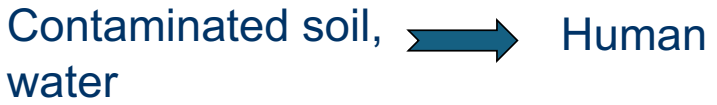
Transmission patterns of infectious diseases

Person-to-person



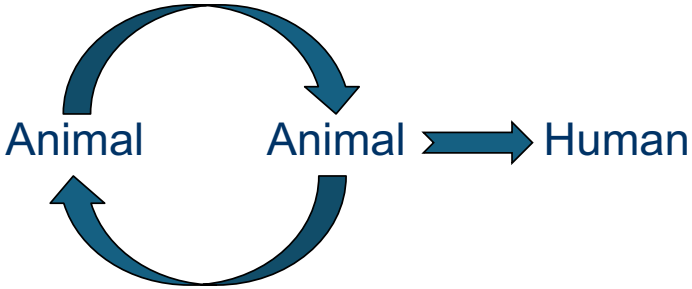
Example: measles, chickenpox

Environment-to-person



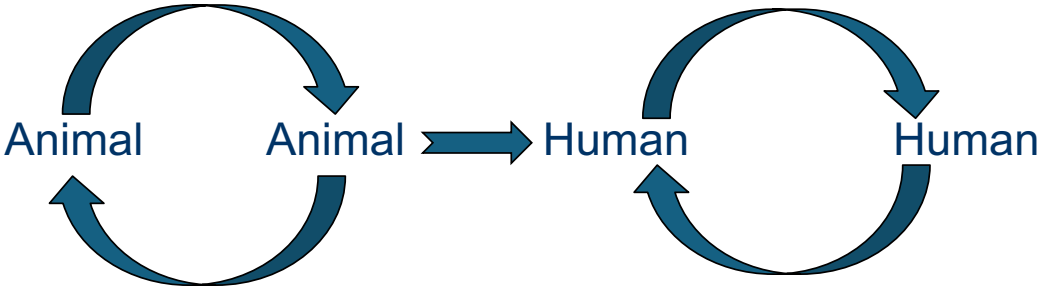
Example: Melioidosis (*Burkholderia pseudomallei*)

Animal-to-person (Zoonosis, spillover)



Example: Rabies virus

Animal-to-person-to-person (Zoonosis -> epidemic/ pandemic)



Example: HIV-1, A(H1N1)pmd09

Modes of transmission

*How does a pathogen travel from a **source (reservoir/ infector)** to a **susceptible host (infectee)**?*

Direct Transmission (mucosal -> mucosal; reservoir -> mucosal)

- **Direct physical contact**: Skin-skin or mucosal-mucosal contacts
- **Direct spray of droplets**: spread via large aerosols that travel within a short-distance
- **Direct contact to an agent in the environment**
- **Vertical (transplacental/ perinatal)**
- **Animal bites**

Indirect Transmission (mucosal -> vector/ vehicle -> mucosal)

- **Airborne**: the infectious agent is spread via fine-size dust or aerosols that may remain suspended in air for an extended time and stay infective.
- **Biological/ vector-borne**: the infectious agent is multiplied/ developed and spread by mosquitoes, fleas, or ticks.
- **Mechanical/ vehicle**: the infectious agent is physically transferred (without multiplication) via food, water, blood, or fomites (inanimate objects such as doorknobs, lift buttons).

PMC7150340

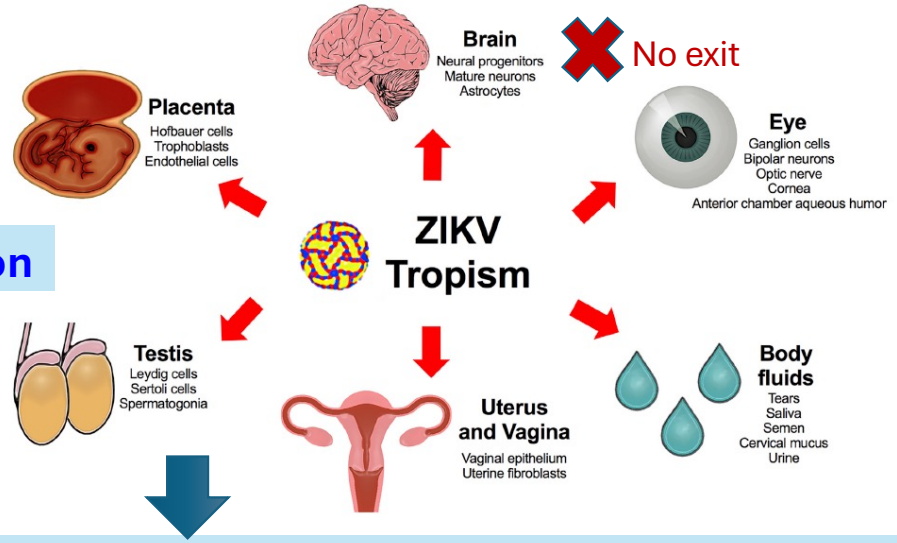
Portals of Entry vs. Portals of Exit

*Targeted by infection control practices to **prevent dispersal** (from the source) & to **protect the portal of entry**.*

Zika virus
Portal of entry



Sites of replication



Portals of exit



Transmission through sex



Vertical transmission



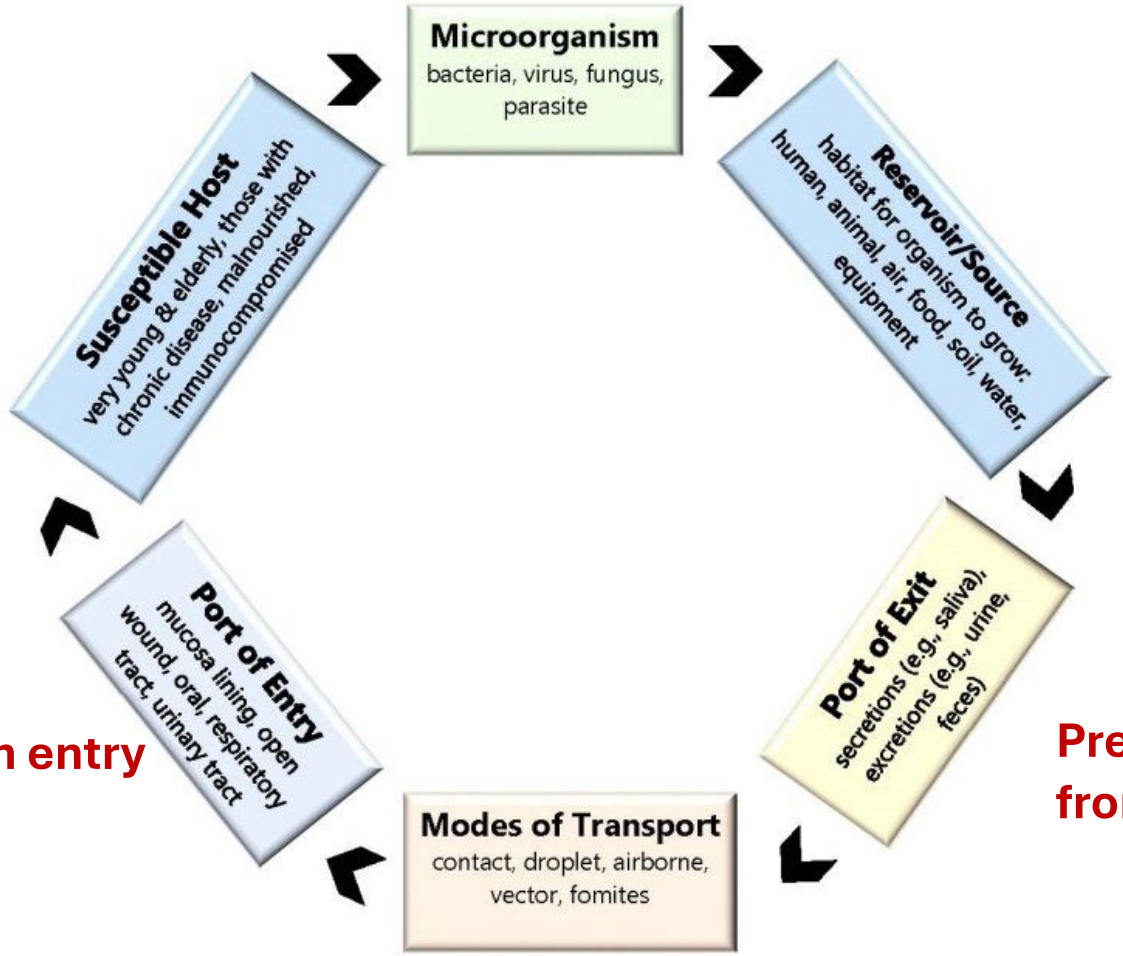
Through blood transfusion



Transmission via vectors

Miner and Diamond. Cell Host Microbe 2017. https://www.cdc.gov/zika/pdfs/Zika-Transmission-Infographic.pdf

Chain of infection

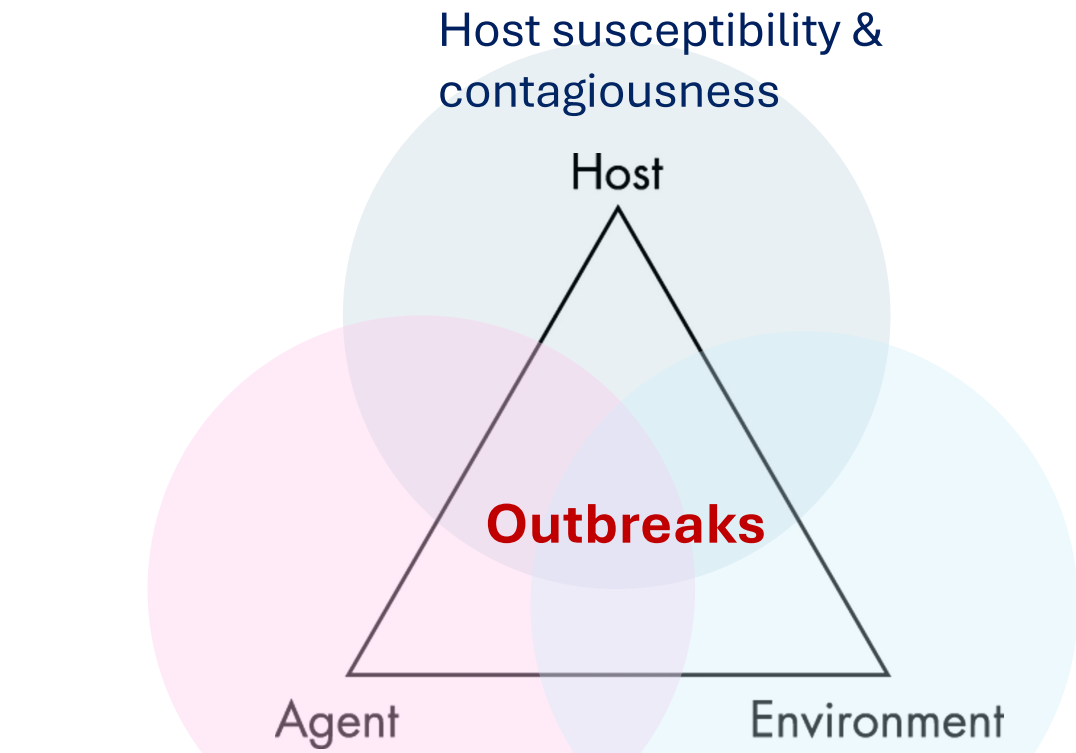


Protect from entry

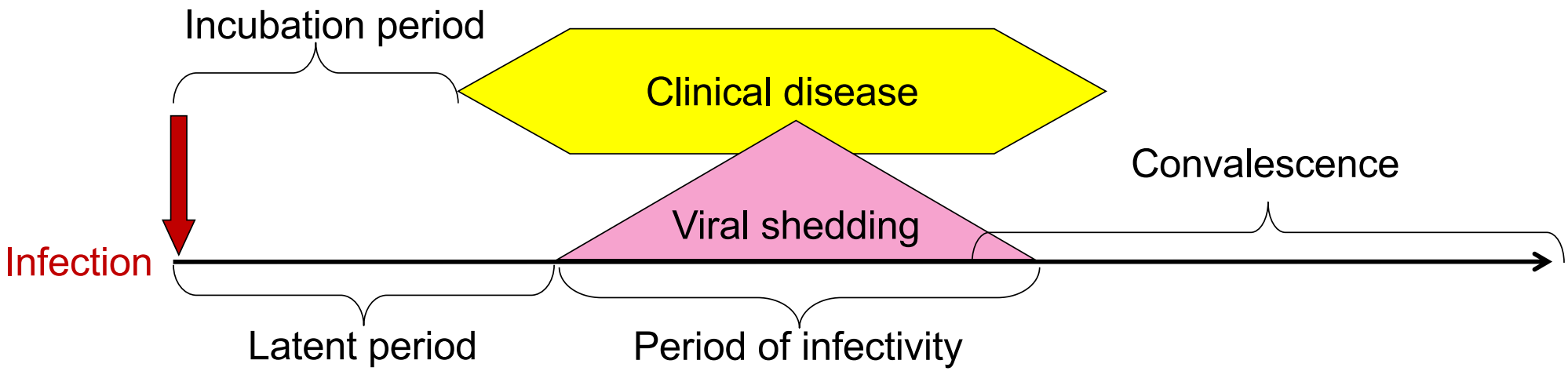
Prevent dispersal from the source

https://www.cdc.gov/niosh/learning/safetyculturehc/module-2/3.html

The epidemiologic triangle describes the agent-host-environment interactions that allow an infectious agent to spread



The time course of infections

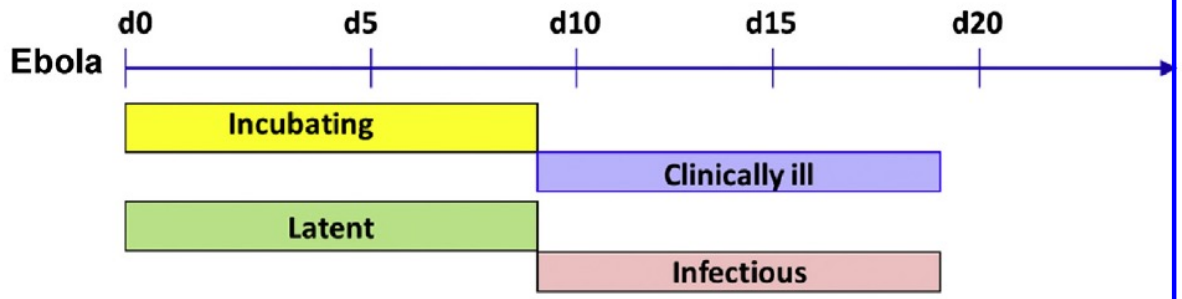


Detection of pathogens

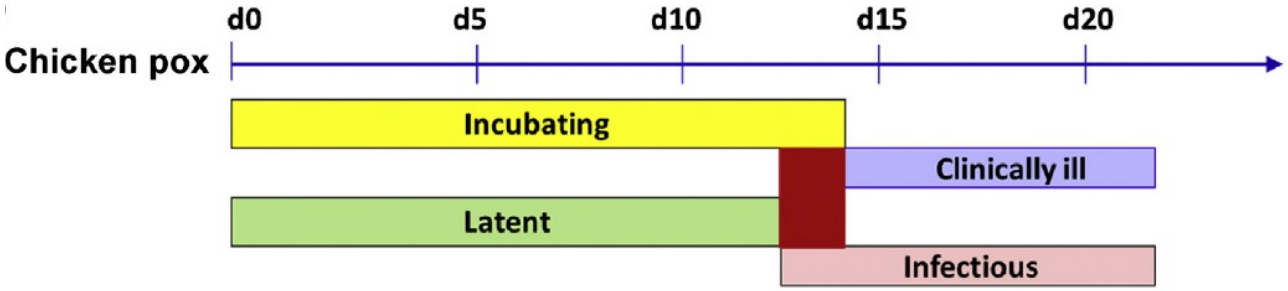
Detection of antibodies

Incubation period vs. latent period

Latent period >= incubation period:
patient is contagious after or at symptom onset



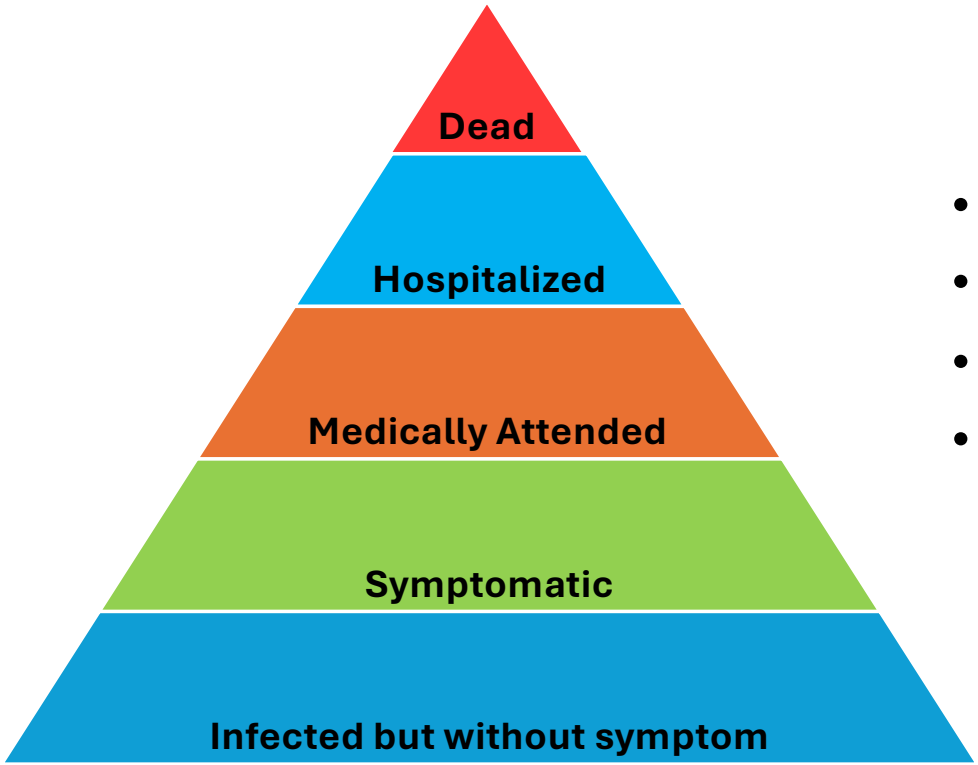
Latent period < incubation period:
patient is contagious before symptom onset



- **Incubation period:** time from exposure to onset of symptoms.
- **Latent period:** time from exposure to onset of contagiousness.

Ease of implementing case identification, isolation, and contact tracing to block transmission chains?

“Clinical iceberg”

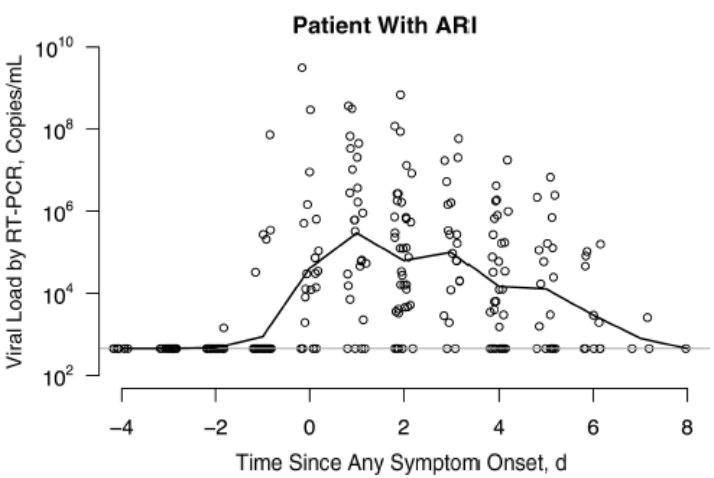


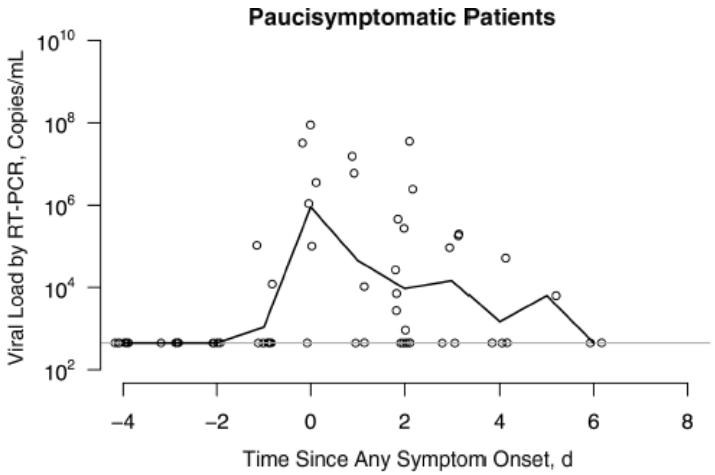
- Age, BMI, pre-existing immunity
- Symptom severity (coughing, fever)
- Pre-symptomatic shedding?
- Viral shedding by pauci-symptomatic or asymptomatic patients?

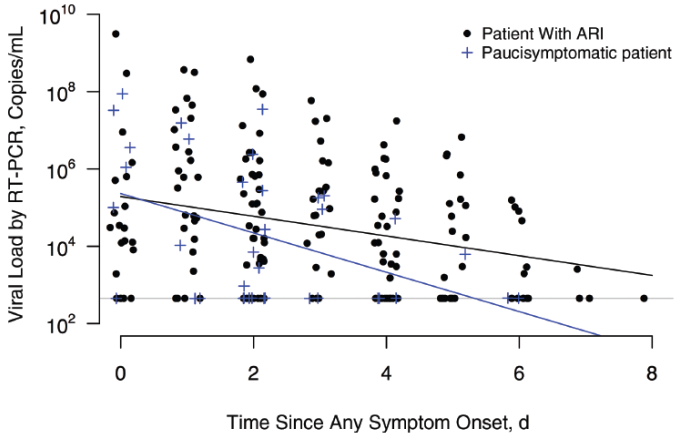
Viral Shedding and Transmission Potential of Asymptomatic and Paucisymptomatic Influenza Virus Infections in the Community

Dennis K. M. Ip,[1] Lincoln L. H. Lau,[4] Nancy H. L. Leung,[1] Vicky J. Fang,[1] Kwok-Hung Chan,[2] Daniel K. W. Chu,[1,3] Gabriel M. Leung,[1] J. S. Malik Peiris,[1,3] Timothy M. Uyeki,[5] and Benjamin J. Cowling[1]

- Household transmission study in HK (2008-2014): following up healthy contacts within households for their daily symptom and viral shedding patterns. **235 secondary cases** were identified by virological test.
- **Viral RNA shedding in asymptomatic and paucisymptomatic cases were approximately 1–2 log10 copies lower than in symptomatic cases.**







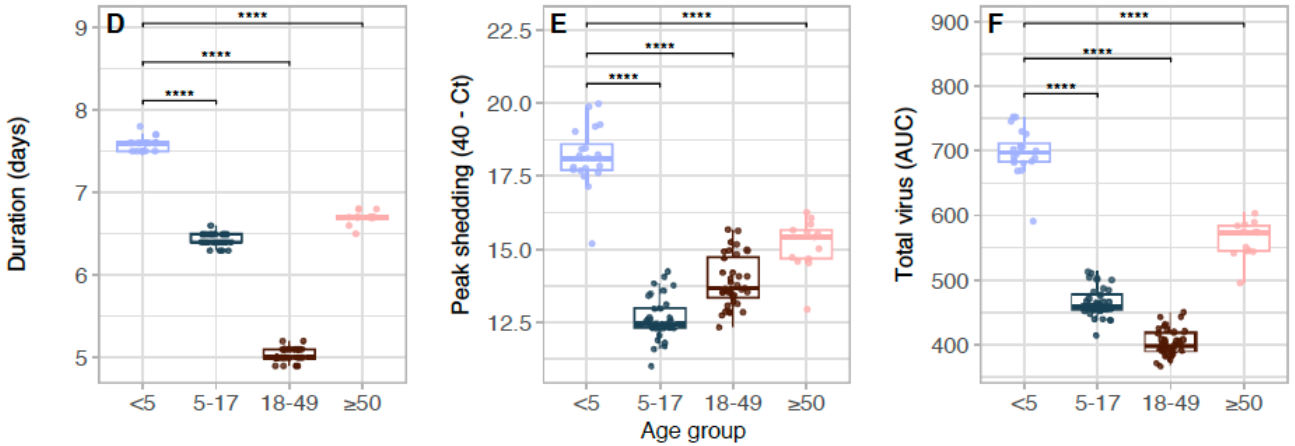
ARI: with ≥2 symptoms (fever ≥37.8 C, headache, myalgia, cough, sore throat, runny nose, and sputum production) ; **pauci-symptomatic: with one symptom**; **asymptomatic: no symptom**

PMID: 28011603

Influenza virus shedding and symptoms: Dynamics and implications from a multiseason household transmission study

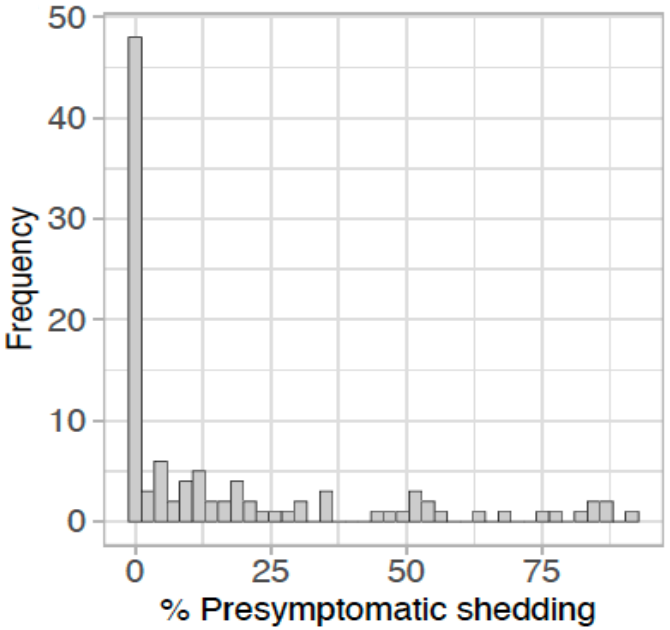
Sinead E. Morris[a,b,*], Huong Q. Nguyen[c], Carlos G. Grijalva[d], Kayla E. Hanson[c], Yuwei Zhu[d], Jessica E. Biddle[a], Jennifer K. Meece[c], Natasha B. Halasa[d], James D. Chappell[d], Alexandra M. Mellis[a], Carrie Reed[a], Matthew Biggerstaff[a], Edward A. Belongia[c], H. Keipp Talbot[d] and Melissa A. Rolfes[a]

Children aged <5 years shed more virus & for a longer period.



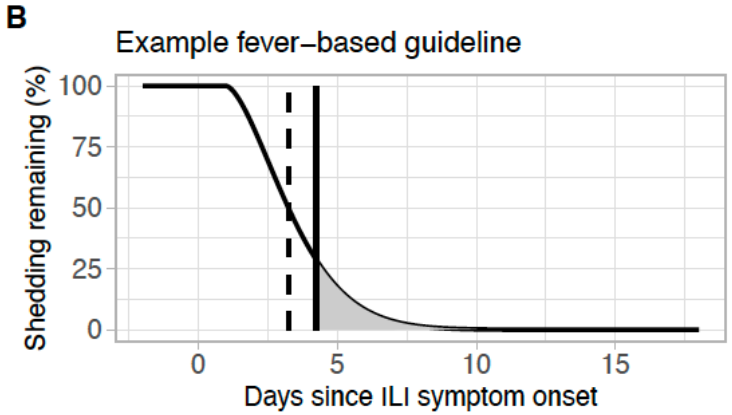
PMID: 39246667

- Of the 116 household contacts with ≥2 positive PCR tests, 105 (91%) experienced ≥1 ILI symptom.
- Among those with at least one ILI symptom, most individuals (60%) shed <10% of their total virus before symptom onset.
- **15% shed more than 50% of their total virus before symptom onset.** No specific factors (age, vaccination, type of virus, etc…) can explain their status.

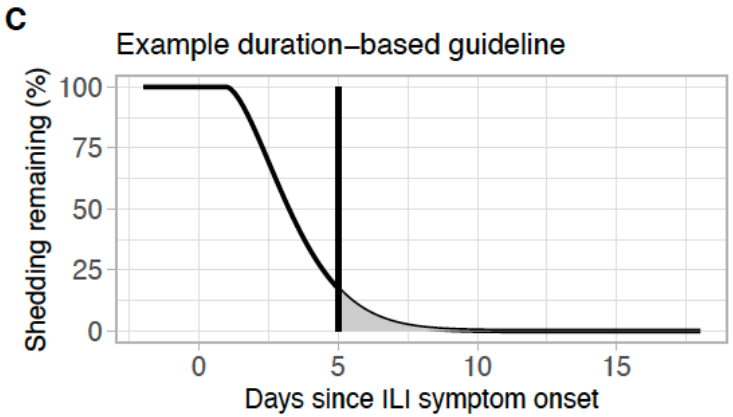


Effect of current isolation guideline

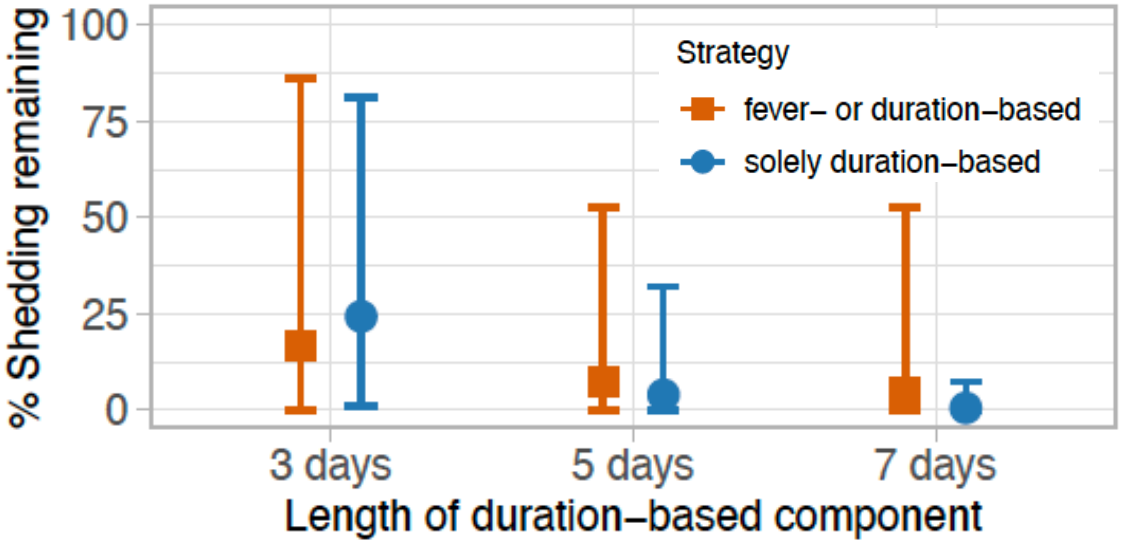
Those with fever: stay at home and avoid contact with others until 24 h after fever resolution



Those without fever: stay at home and avoid contact with others for 5 days following symptom onset



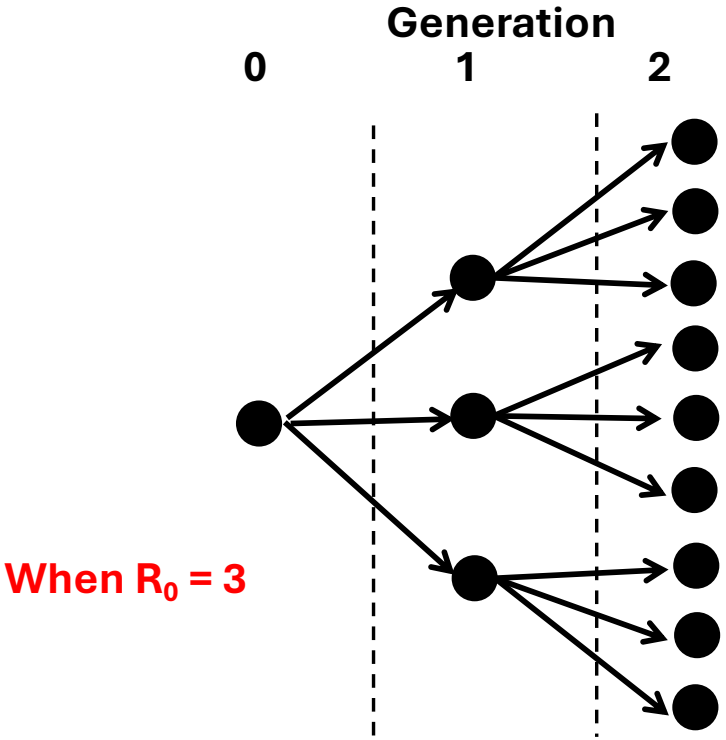
Shedding may be remained in 16.5%, 7.5%, or 4.6% individuals (median percentage) after 3, 5, or 7 days of isolation, respectively.



PMID: 39246667

Basic reproduction number (R_0)

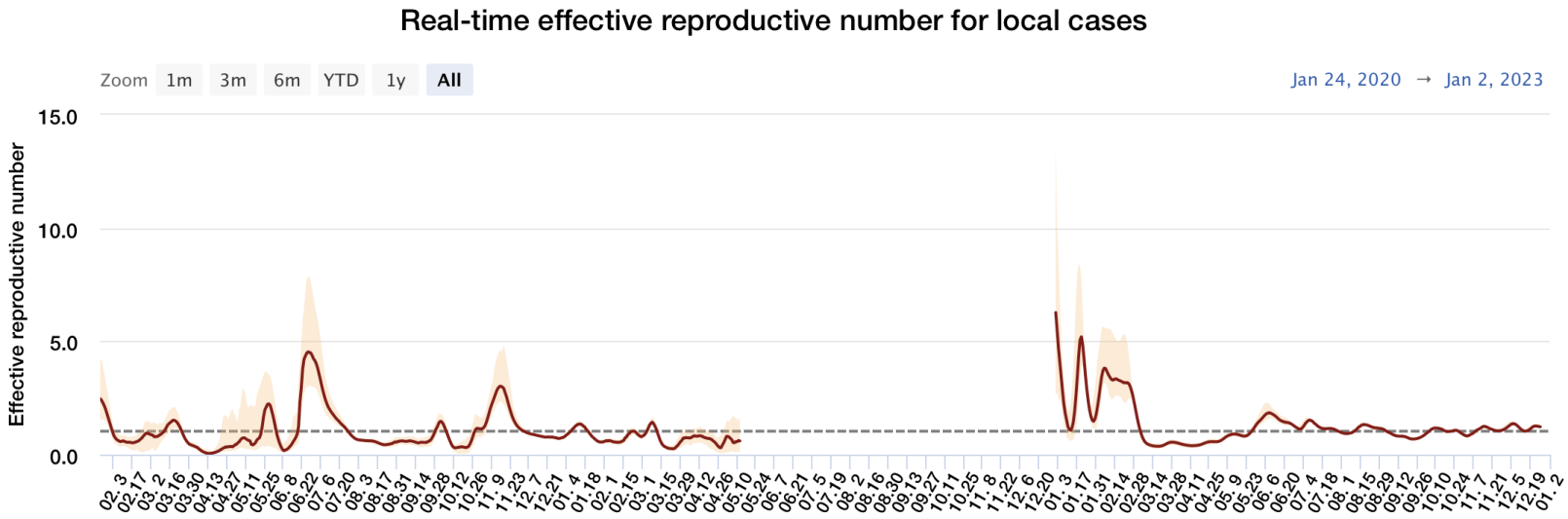
Basic reproduction number (R_0): The average number of **secondary infections** caused by **an average infected individual** in a **susceptible population** (eg. no herd immunity and no intervention was implemented). For a disease to spread, R_0 needs to be greater than 1.



Effective reproduction number (Rt)

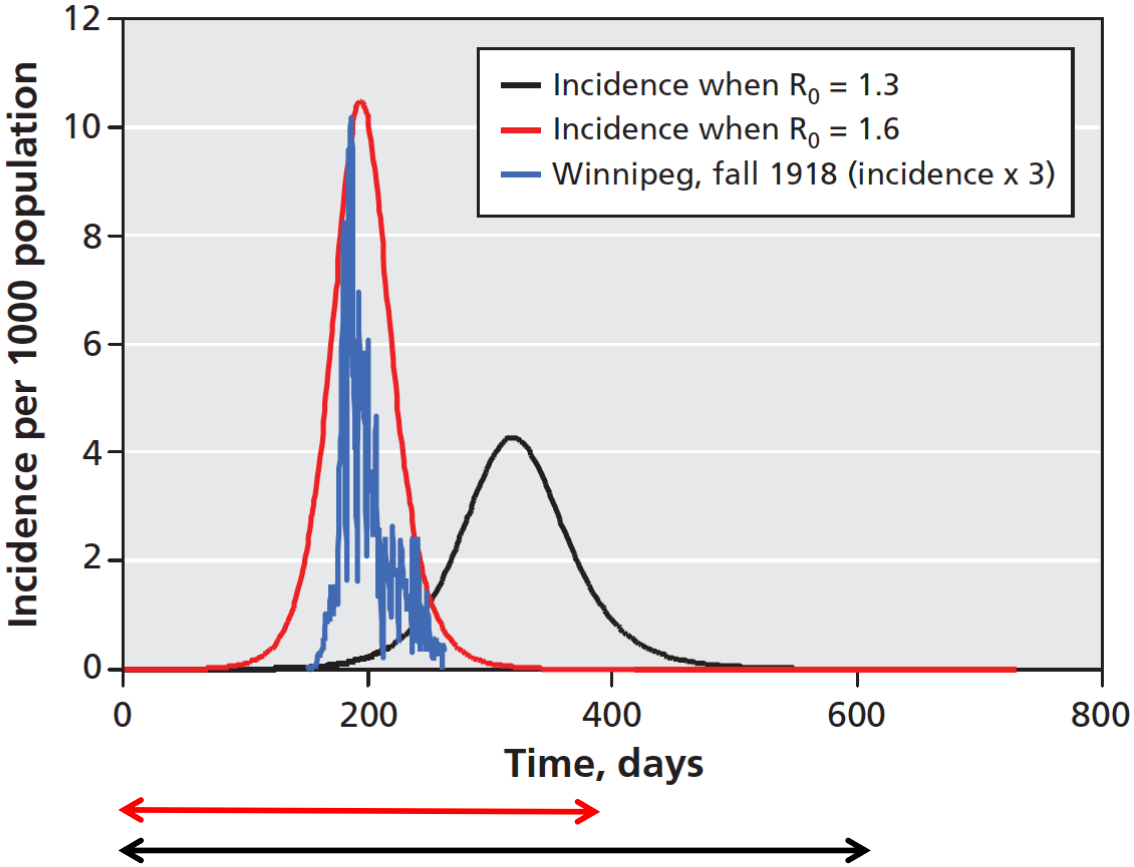
Effective reproduction number (R_t) at a given time: The average number of secondary cases per one case **at a given time (t)**. R_t shows **time-dependent variation** due to implementation of **effective control measures, behavioral changes, & population immunity**.

- If **R_t <1**, it suggests that the epidemic is in decline and may be regarded as being under control **at time t**.
- If **R_t >1**, each infected patient will go on to infect on average more than one person **at time t**.



https://covid19.sph.hku.hk/dashboard

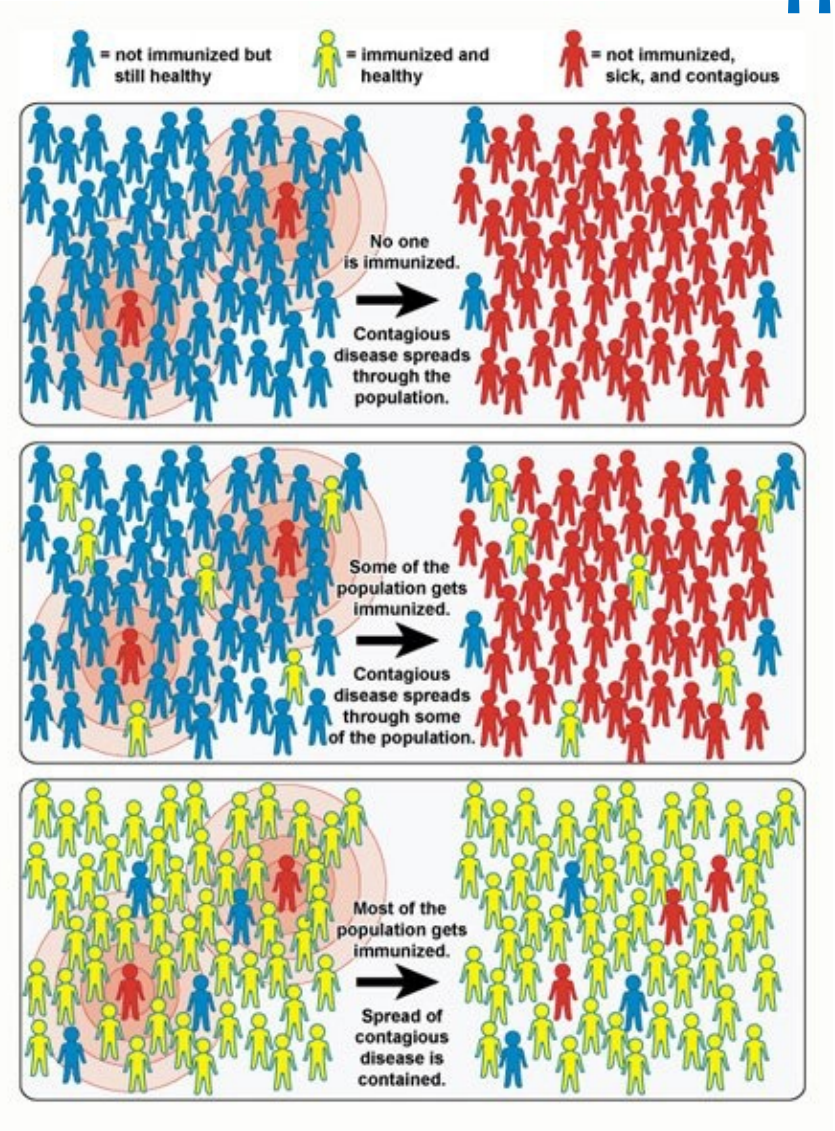
The effect of changing the effective reproductive number on the severity and duration of an influenza epidemic



Public health interventions may affect effective reproductive number (Rt) -> **flatten the curve.**

CMAJ 2009;181:171-173

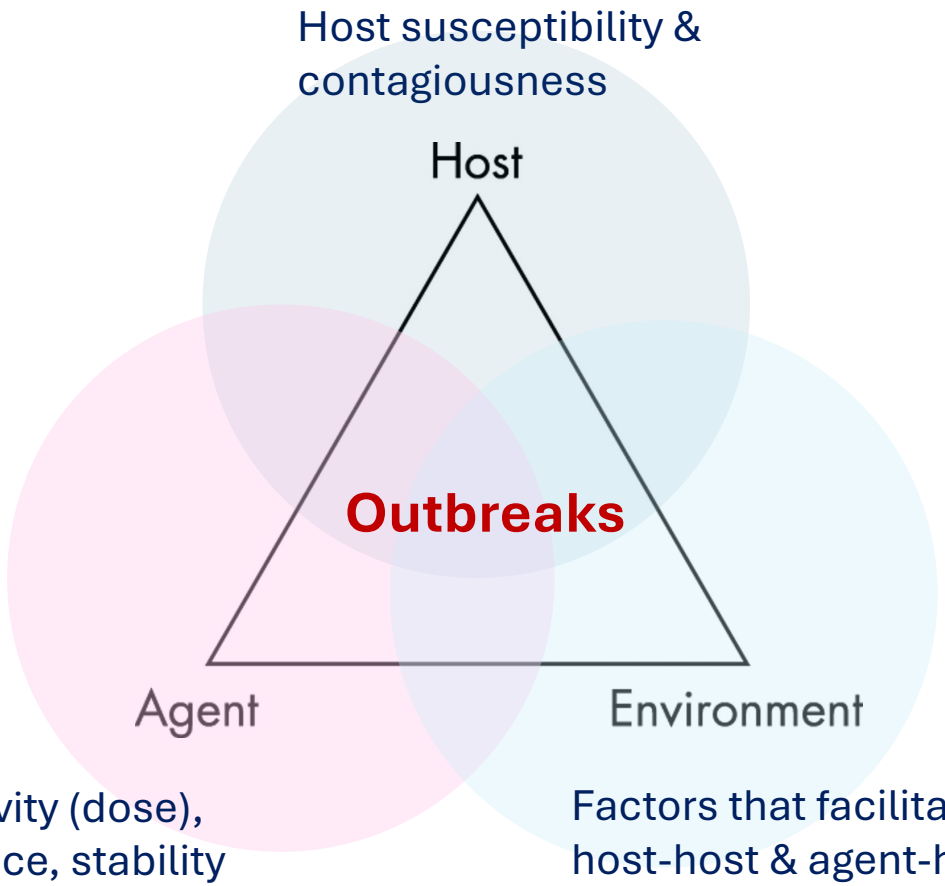
Herd immunity



- The risk of infection among susceptible individuals in a population is reduced by the presence and proximity of immune individuals (**indirect protection or herd effect**).
- **Herd immunity threshold = $[(R_0-1)/R_0]$.** A threshold proportion of immune individuals within a population that lead to a decline in disease transmission and infection risk (**Rt <1**).
- If vaccination leads to **perfect immunity** then the **critical vaccination level** (V_C) = herd immunity threshold = $[(R_0-1)/R_0]$. If vaccine effectiveness (*VE*) is not 100%, then $V_C = [(R_0-1)/R_0]/ VE$

Fine, Eames, and Heyman. 2011. CID 52:911-916. Gani and Leach. 2011 Nature. 414:748–51. Gani and Leach. 2012 Nature. 415:1056.

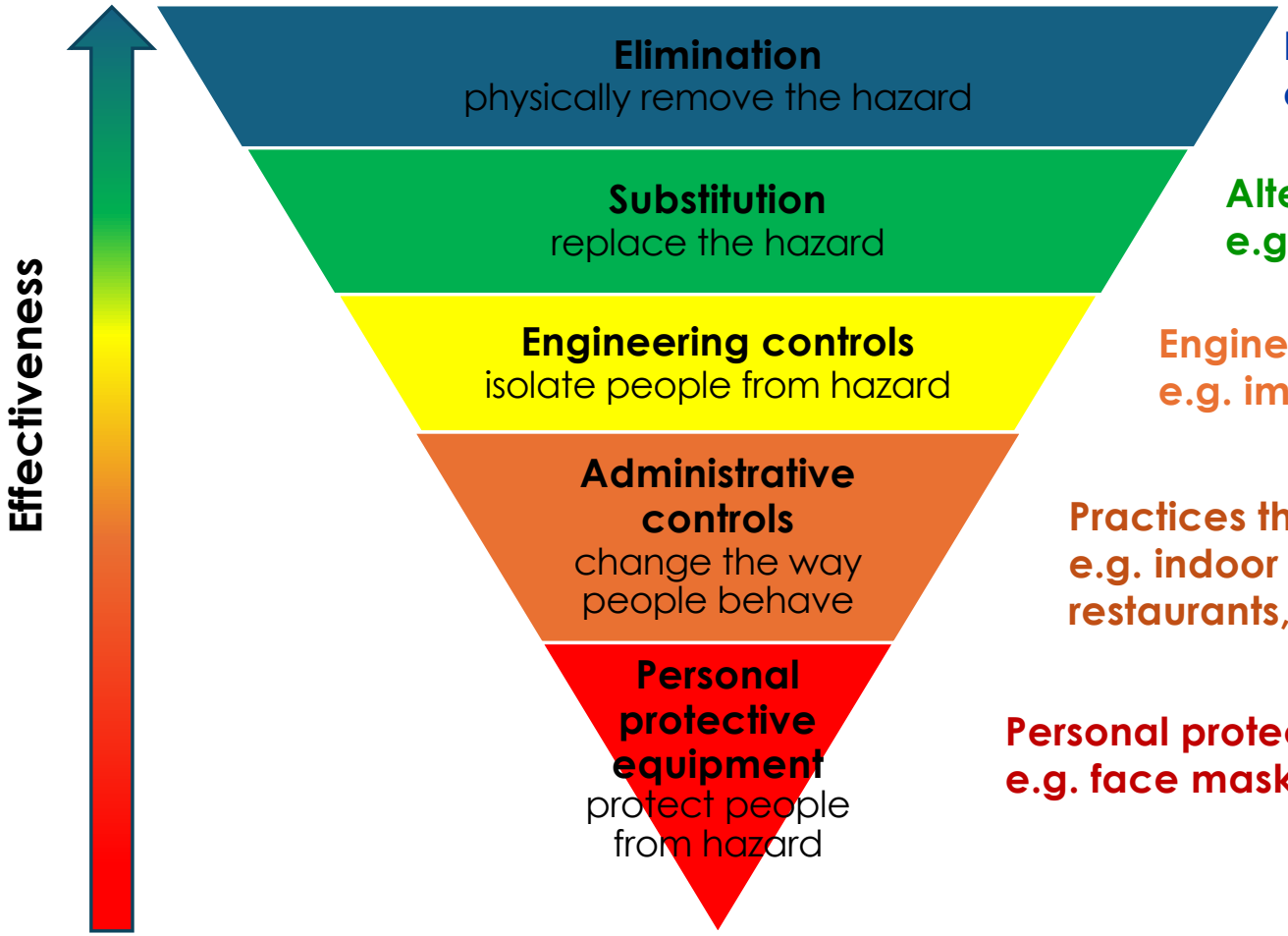
Intervention: breaking the epidemiologic triangle



- The agent's reservoir or source
- The mode of spread or transmission
- Host-related risk factors
- Environmental and other mediating factors
- A priori evidence of effectiveness of the intervention
- Operational and logistical feasibility
- Legal authority necessary to support implementing the measure

Hierarchy of hazard control

Risk management for (occupational) safety and health



Prevent contact with infectious agents:
e.g. isolation & quarantine

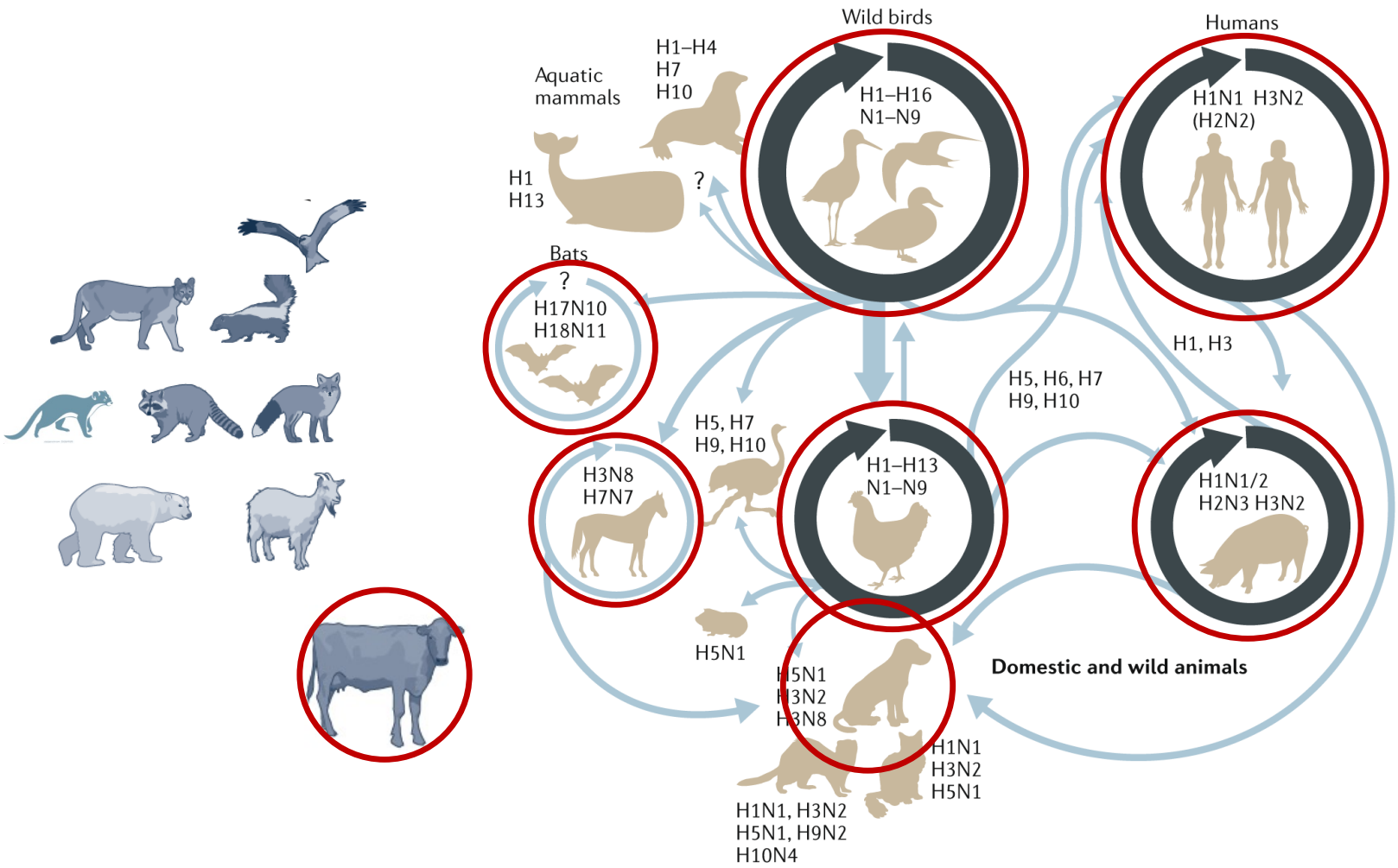
Alternative measures for elimination:
e.g. work from home, outdoor activities

Engineering protective barriers:
e.g. improve ventilation, filtration of air

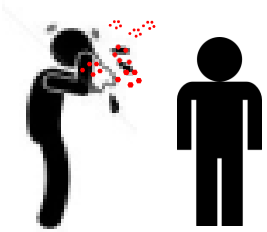
Practices that reduce the risk of exposure:
e.g. indoor density, four-person per table at restaurants, mask mandate, daily RAT

Personal protection:
e.g. face mask, respirators, face shield

Hosts that support sustained transmission vs. spillover hosts of influenza A viruses

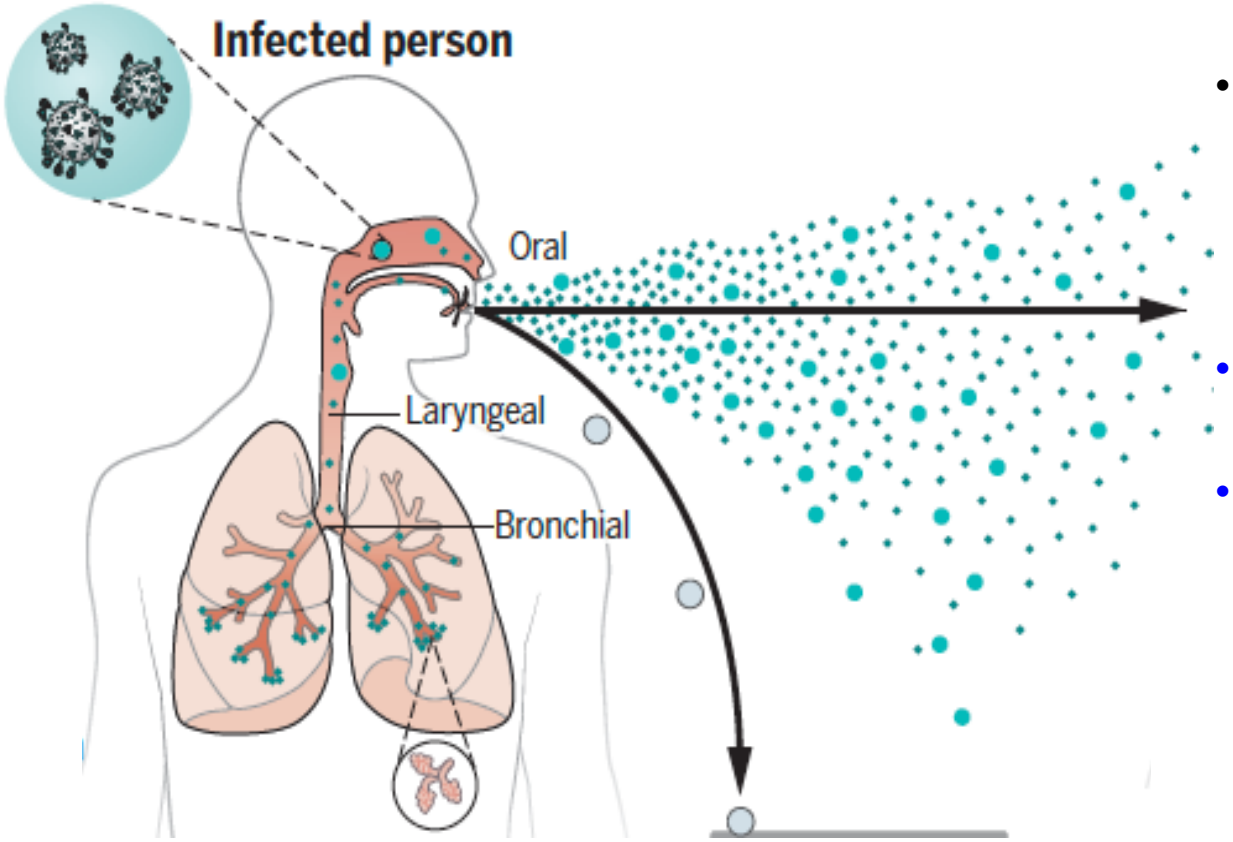


PMID: 30487536
PMID: 39317240



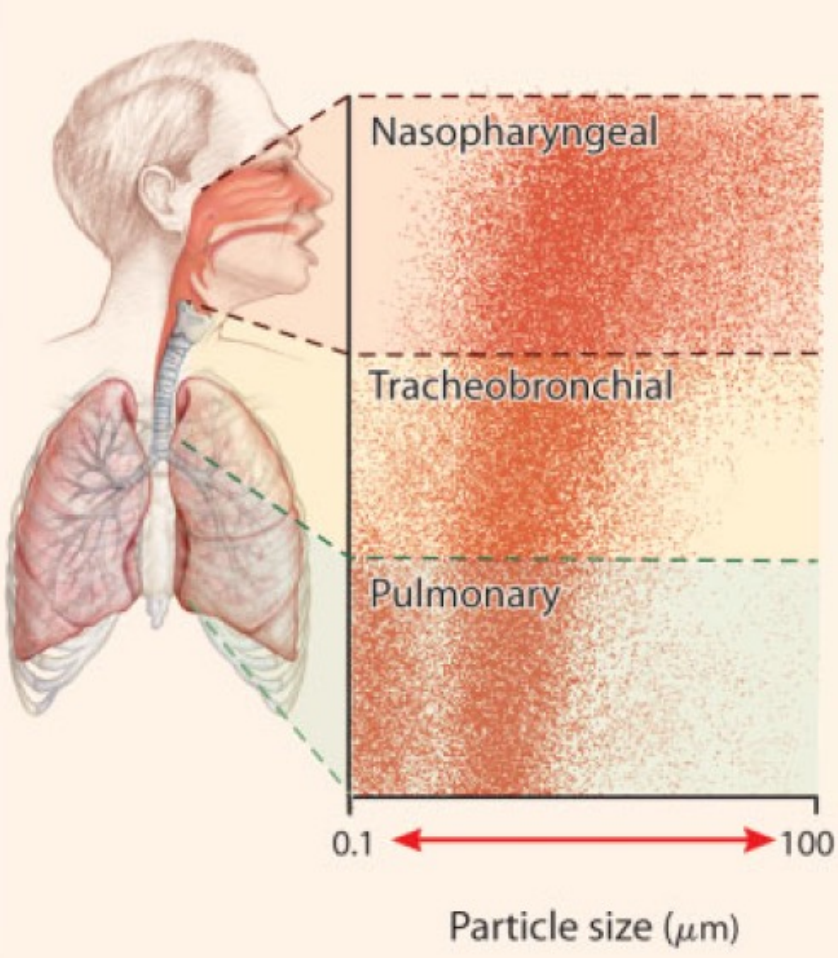
Influenza transmission in humans

Portal of exit



- Virus-laden particles (**infectious respiratory particles, IRPs**) may be **exhaled** (via breathing) or **expelled** (via coughing, speaking, etc…) from an infected person.
- **Exhaled or expelled IRPs exist as a continuum of particle size.**
- **Pending on the size, IRPs show different spatial-temporal distribution.**
  - Large IRPs: deposit within a short range and within a short time frame.
  - Fine IRPS: may remain suspended in air and may travel a longer distance.

Portal of entry

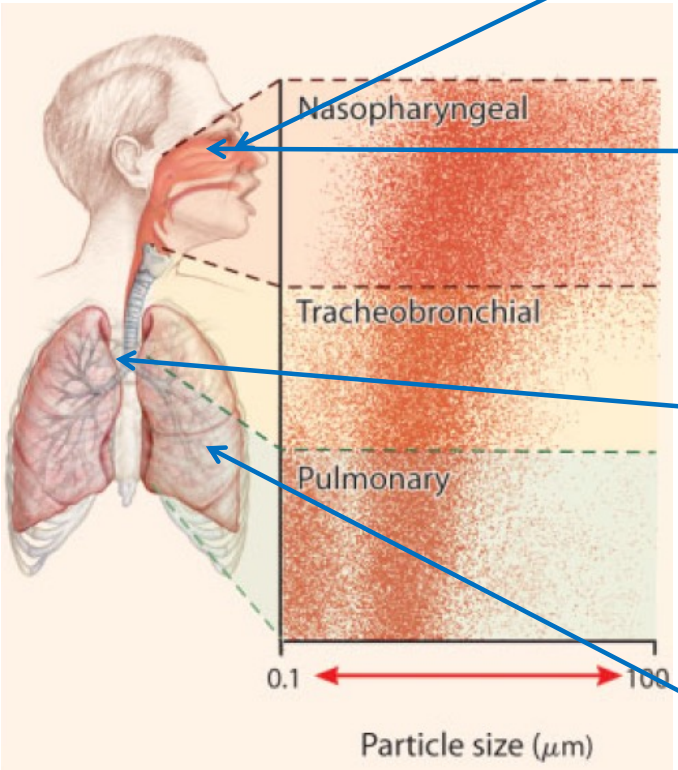


- **Large IRPs (semi-ballistic sprays)** may be **deposited** at the exposed mucosal surfaces (eg. eyes) of a susceptible host nearby.
- **Fine IRPs (< 100 μm)** may be **inhaled** and reach respiratory mucosal surfaces of a susceptible host (nearby or with a distance).
  - Pending on the size, fine IRPs may reach different regions of the respiratory tract. In general, particles <5 μm may reach the lungs.
  - Fine IRPS also can be deposited at the exposed mucosal surfaces.
- **Direct contact or indirect contact via fomites**: viruses may be transferred to the respiratory or ocular mucosal surfaces.

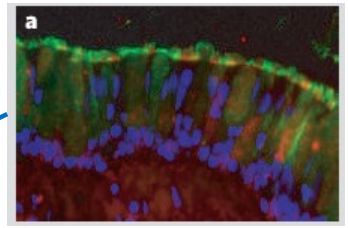
Roy and Milton. NEJM. 2004

Receptor distribution and tropism

SNA lectin: detect α2,6-linked SA
MAA lectin: detect α2,3-linked SA

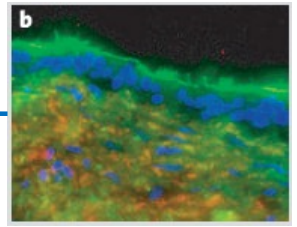


Nasal mucosa



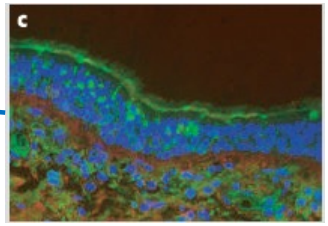
α2,6: ++
α2,3: +

Paranasal sinuses



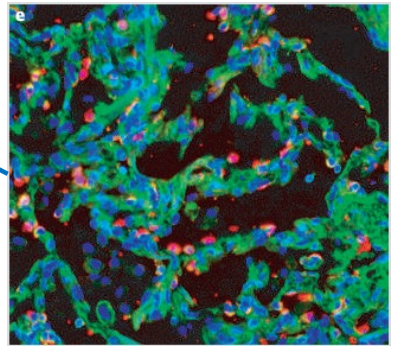
α2,6: +++

Bronchus



α2,6: +++

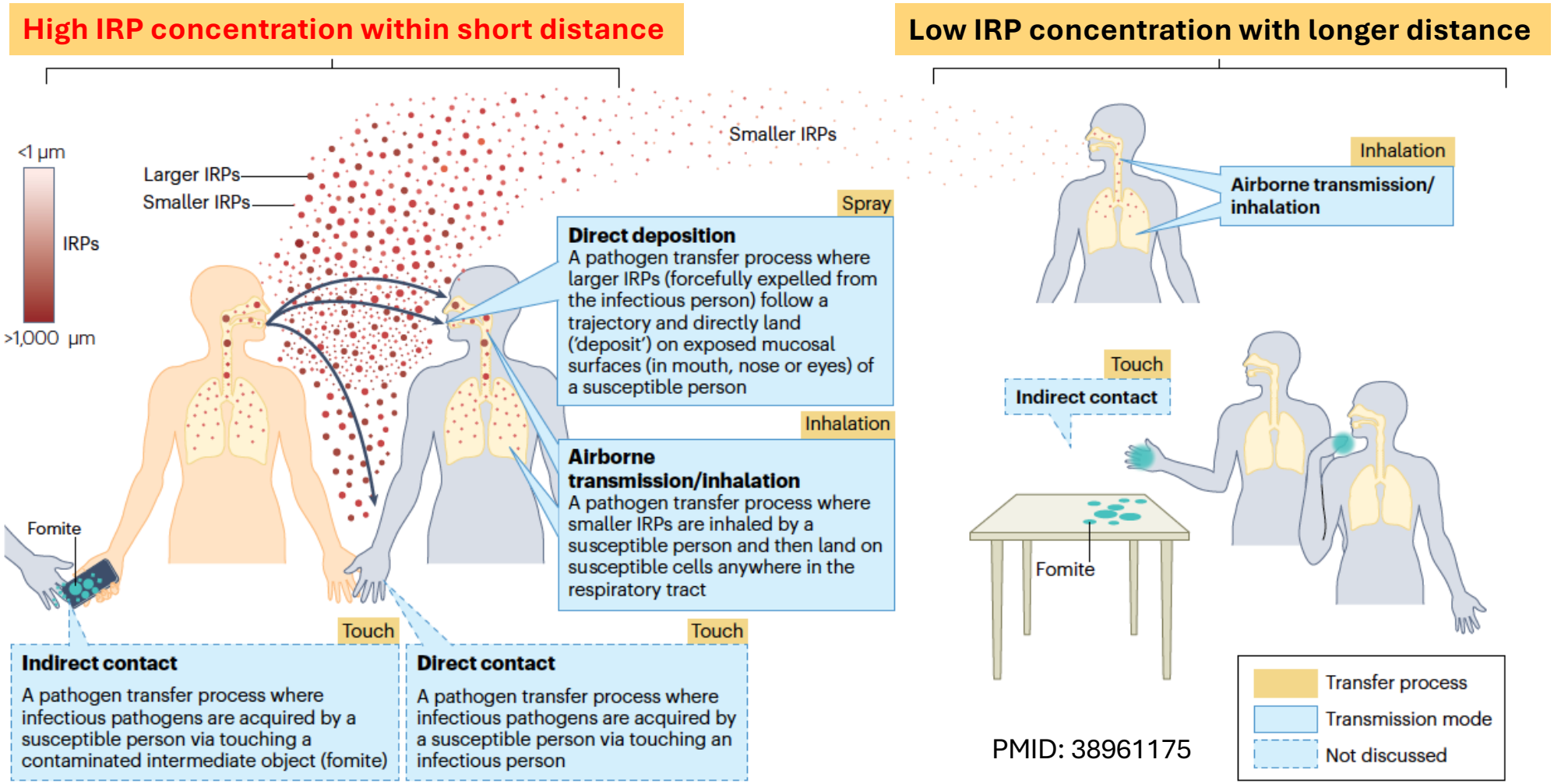
Alveoli



α2,3: ++
α2,6: +

Roy and Milton, NEJM 2004.
Shinya et al., Nature 2006.

Multiple & non-mutually exclusive transmission modes



PMID: 38961175

Studies that support transmissions via IRPs

A VIRUS OBTAINED FROM INFLUENZA PATIENTS

By Wilson Smith, M.D. Manch.
C. H. Andrewes, M.D. Lond.
and
P. P. Laidlaw, B.Chir. Camb., F.R.S.

(From the National Institute for Medical Research, Farm Laboratories, Mill Hill)

The epidemic of influenza at the beginning of 1933 afforded an opportunity of making an experimental study of this disease, the results of which are here embodied in a preliminary communication. Throat-washings were obtained from a number

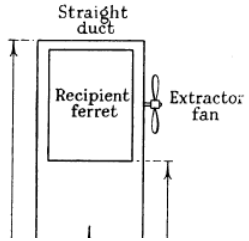
The first human influenza virus isolate was transmissible among ferrets.

ferret was used and the first success was only secured towards the close of the epidemic.

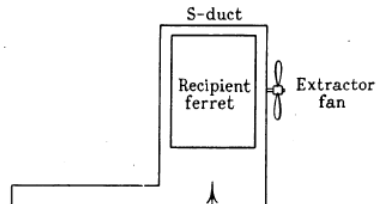
The initial successful experiment was made with two ferrets, both of which received a filtrate of human throat-washings, both subcutaneously and by intranasal instillation. Both animals became obviously ill on the third day after infection and exhibited symptoms of the characteristic disease which is described below. It was found that the disease could be transmitted either by contact or by direct transference of nasal washings from a sick to a healthy ferret. At this point therefore the work was transferred to the Institute's farm laboratories at Mill Hill, where it could be carried out under the conditions of rigid isolation of individual experimental animals evolved and used by Dunkin and Laidlaw [1] in their work on dog distemper * (1926).

Experimental evidence to support the role of IRPs in transmission

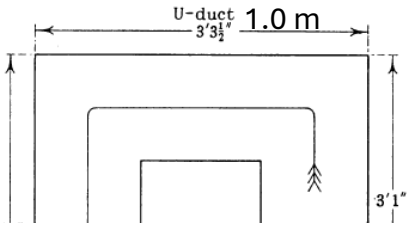
3/3 (3, 13, and 14 ft/min)



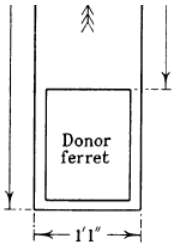
1/1 (14 ft/min)



2/2 (3 and 13 ft/min)



But the specific IRPs size range mediating transmission was not clear.



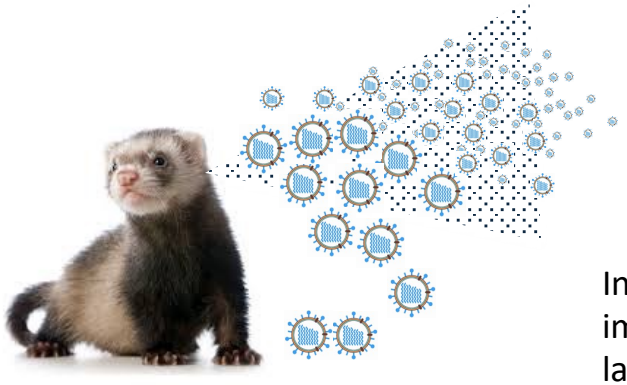
1′1″

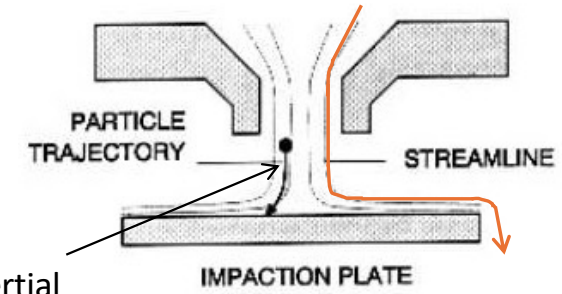
TABLE II.—*Experiments with Ducts.*

ient	No. of recipient ferret.	Duct used.	ft./min. Air-speed	Result.	Incubation period in days.
	129	Straight	14	+	6 (?)
	130	S—	14	+	3
13	159	Straight	3	Mild take, immune later	5
	160	U—	3	Do.	7
14	177	Straight	13	+	8
	178	U—	13	+	6

Andrewes and Glover. The British Journal of Experimental Pathology 1941

The use of impactors to separate large and fine aerosols by size

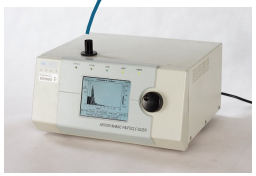




Inertial impaction for large particles

Small particles travel with air flow

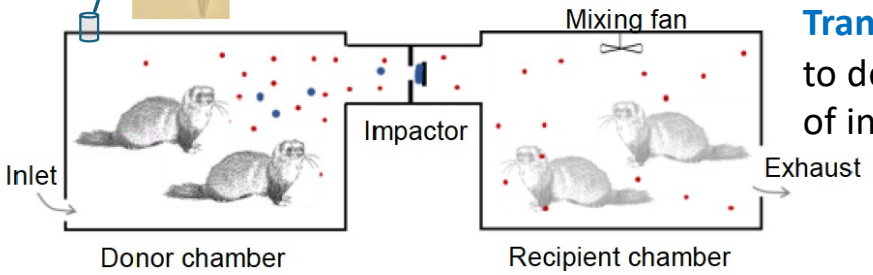
Collection efficiency		
5% (d5)	**50% (d50)**	**95% (d95)**
5.4 µm	9.9 µm	15.3 µm
2.9 µm	5.3 µm	7.9 µm
1.0 µm	2.5 µm	4.3 µm
0.6 µm	1.0 µm	1.5 µm





NIOSH bioaerosol sampler:
to measure size distribution of influenza-laden aerosols

Aerodynamic particle sizer:
to monitor size distribution of total exhaled particles

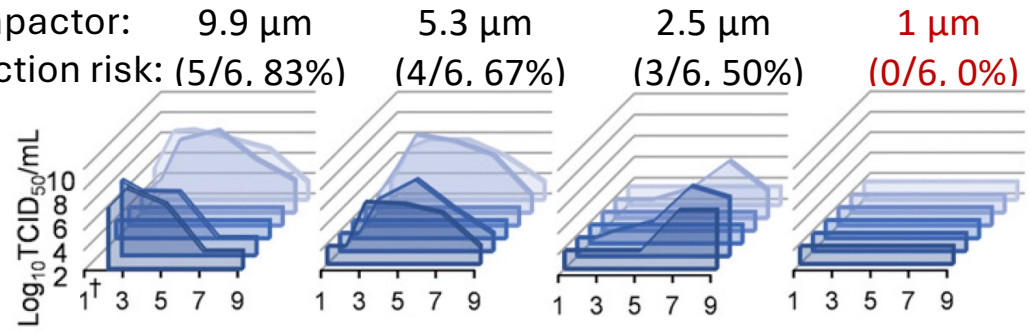


Transmissibility to ferrets:
to determine size distribution of infectious aerosols

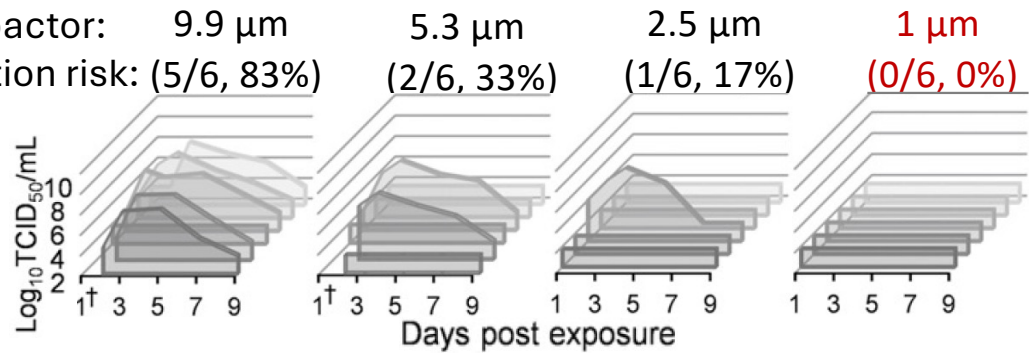
Zhou and Wei et al. *Proc Natl Acad Sci U S A*. 2018;115(10):E2386-E2392.

Infectious influenza viruses that mediate transmission between ferrets were found in breath aerosols > 1.5 µm

A(H1N1)pdm09

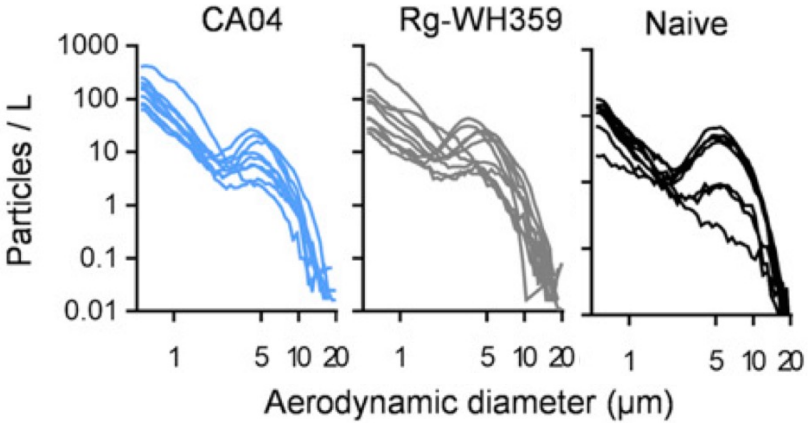


A(H3N2)



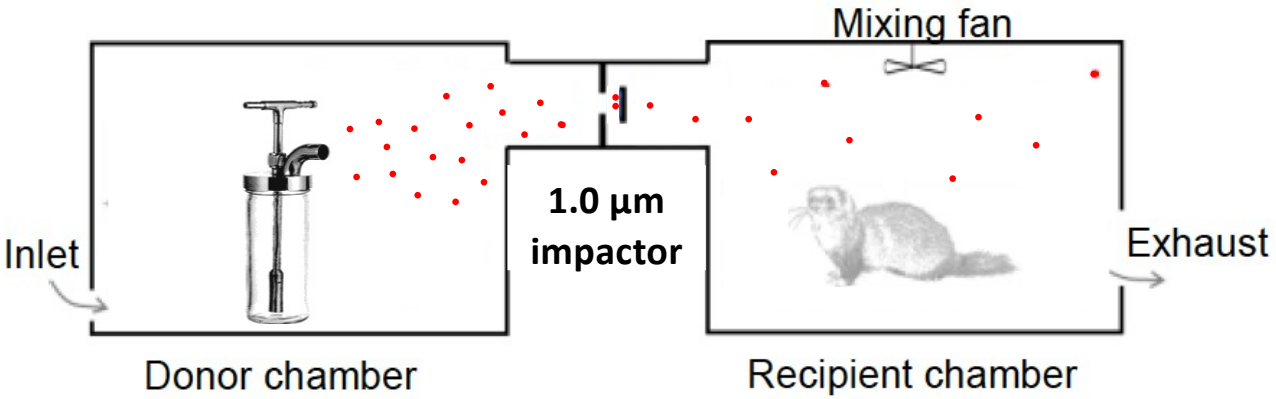
Collection efficiency		
5% (d5)	**50% (d50)**	**95% (d95)**
5.4 µm	9.9 µm	15.3 µm
2.9 µm	5.3 µm	7.9 µm
1.0 µm	2.5 µm	4.3 µm
0.6 µm	1.0 µm	1.5 µm

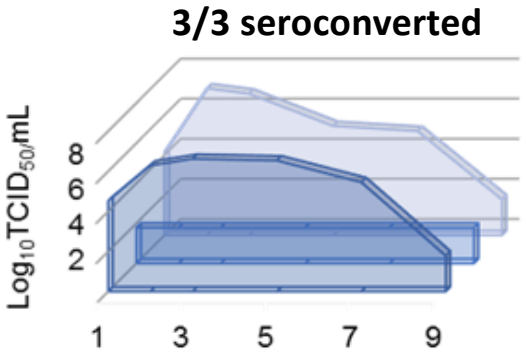
77% of exhaled particles are ≤ 1.5 µm



Aerosol size restriction for packing infectious virions?

Influenza may be transmitted via artificially generated fine aerosols <1.5 µm

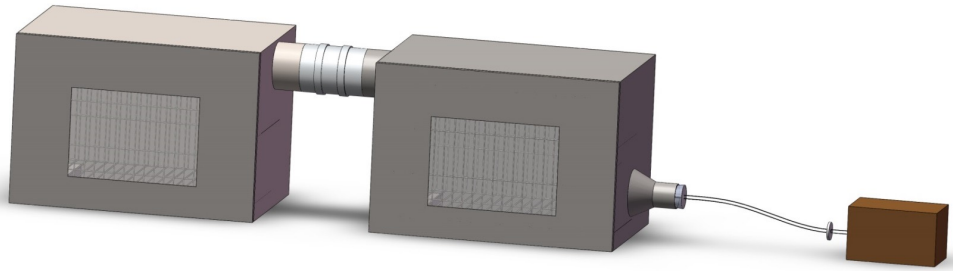




Collection efficiency of the impactors		
5% (d5)	**50% (d50)**	**95% (d95)**
5.4 µm	**9.9 µm**	**15.3 µm**
2.9 µm	**5.3 µm**	**7.9 µm**
1.0 µm	**2.5 µm**	**4.3 µm**
0.6 µm	**1.0 µm**	**1.5 µm**

Majority of the infectious influenza viruses are packaged in breath aerosols > 1.5 µm.

Assessing the communicable period of CA04-inoculated ferrets



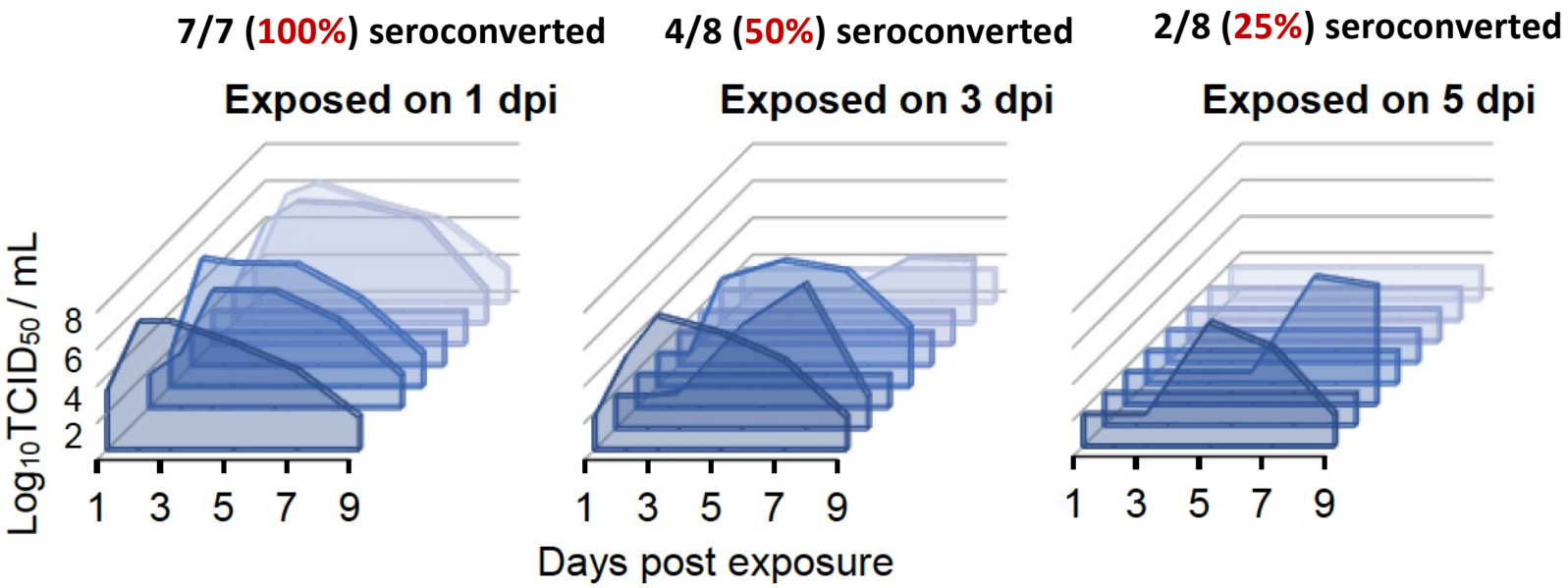
D0
Inoculation of donors

D1
Exposure of recipients #1, #2

D3
Exposure of recipients #3, #4

D5
Exposure of recipients #5, #6

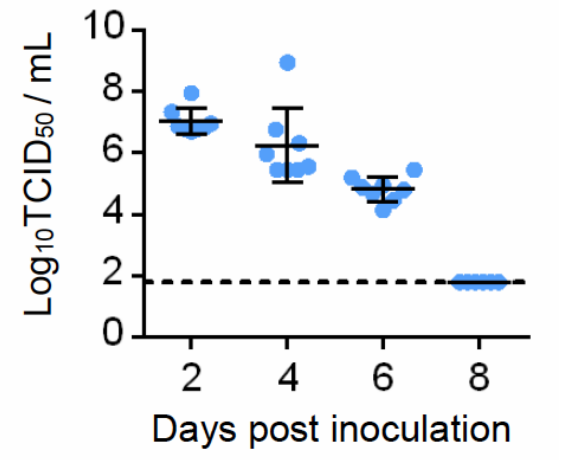
Transmissibility of CA04-inoculated ferrets decreases over time and may last at least five days



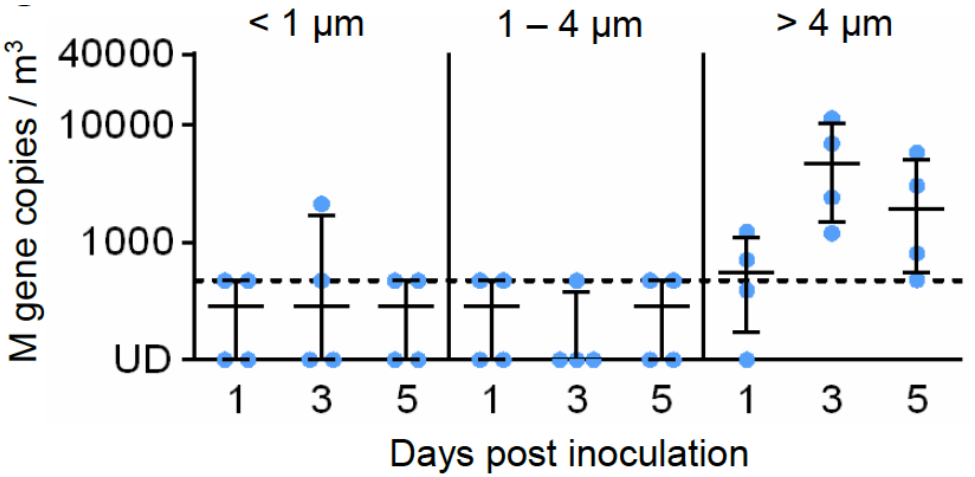
The 5.3 μm (d95 = 7.9 μm) impactor was used.

CA04-inoculated donors continued to release virus-laden particles in air from day 1 to 5 post-inoculation

Ferret nasal wash titers



Quantity and size of virus-laden particles detected from donors



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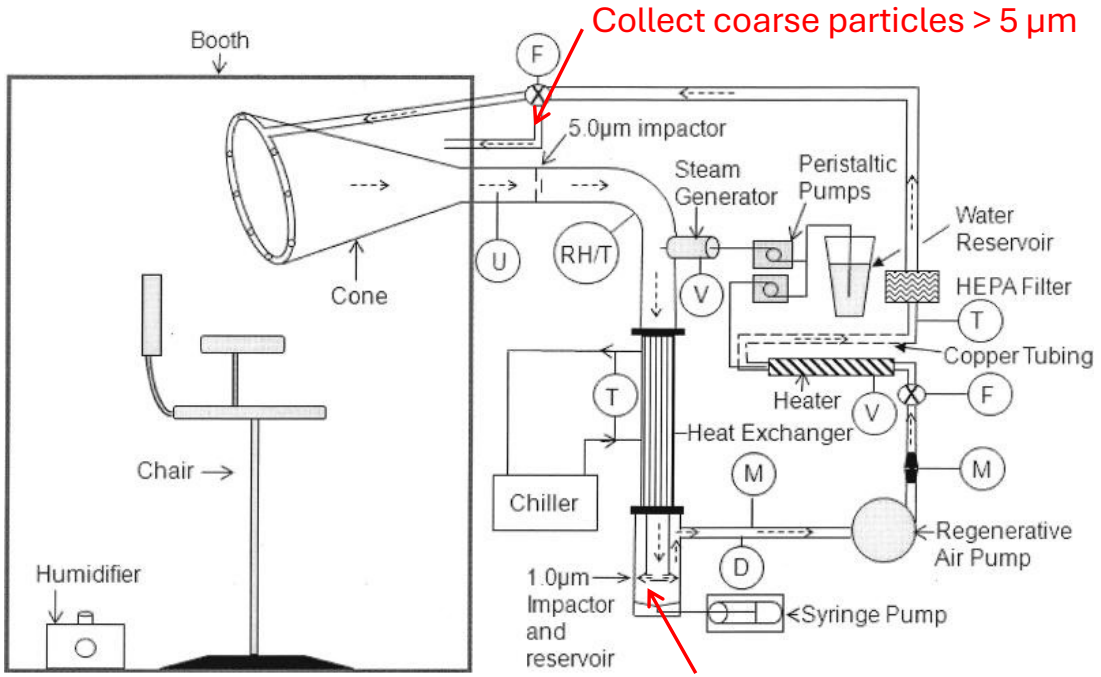
Influenza Virus Aerosols in Human Exhaled Breath: Particle Size, Culturability, and Effect of Surgical Masks

Donald K. Milton[1,2]*, M. Patricia Fabian[2,3], Benjamin J. Cowling[4], Michael L. Grantham[1], James J. McDevitt[2]

1 Maryland Institute for Applied Environmental Health, University of Maryland School of Public Health, College Park, Maryland, United States of America, **2** Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, United States of America, **3** Department of Environmental Health, Boston University School of Public Health, Boston, Massachusetts, United States of America, **4** Department of Community Medicine and School of Public Health, Li KaShing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Enrollment:
Fever with cough within 72h, positive for Flu A/B detection in nasopharyngeal specimen by rapid test (N=33) and by PCR (N=38).

Breath with and without face mask (N=37).



Milton et al., PLoS Pathog. 2013;9(3):e1003205.

Surgical masks effectively reduced the coarse virus-laden particles (≥ 5µm) released from breath

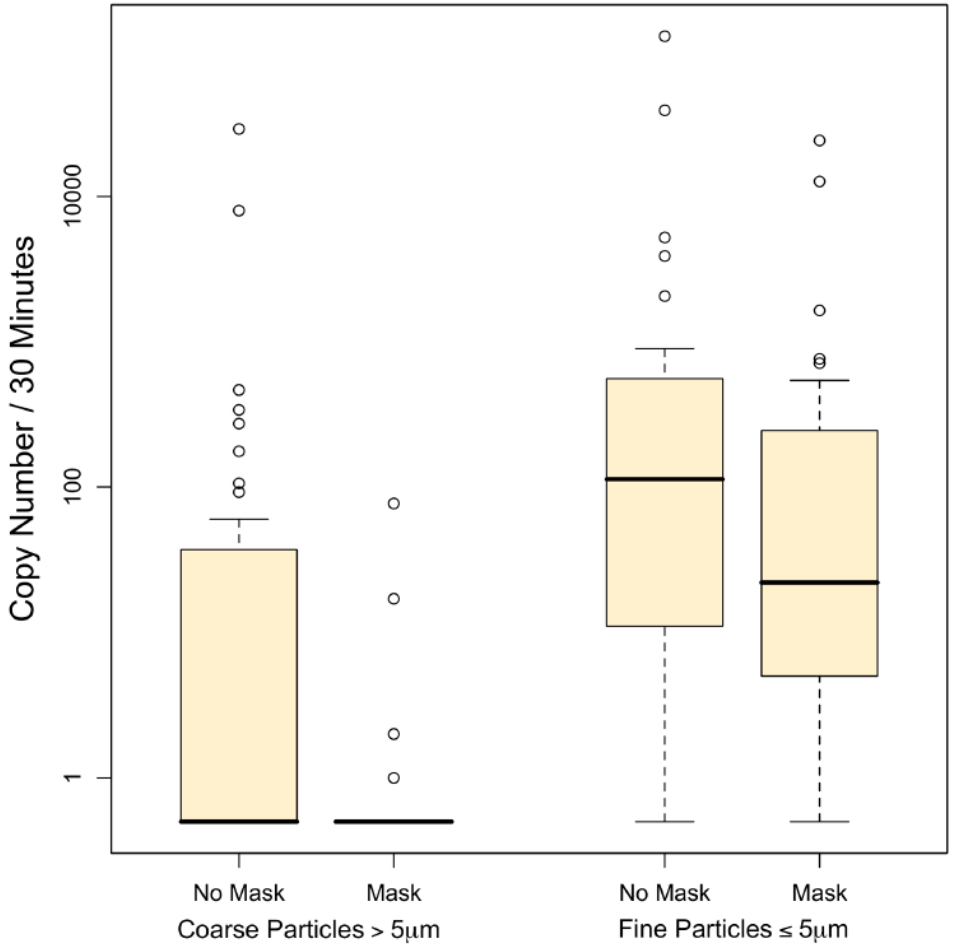


Figure 1. Influenza virus copy number in aerosol particles exhaled by patients with and without wearing of an ear-loop surgical mask. Counts below the limit of detection are represented as 0.5 on the log scale.
doi:10.1371/journal.ppat.1003205.g001

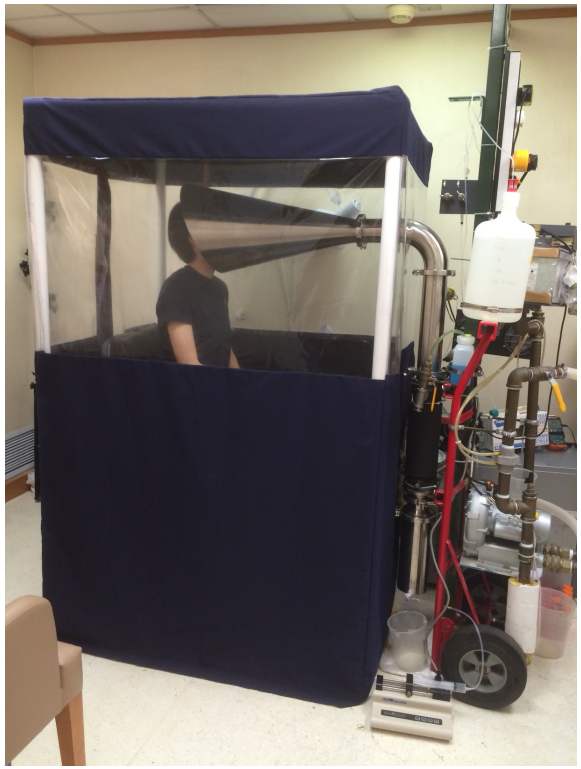
- Higher viral RNA copies detected in fine particles (≤ 5 µm) than in coarse (> 5 µm) aerosol particles.
- Surgical masks showed more reduction in the coarse particles (25-fold) than that of the fine particles (2.8-fold).
- Infectious virus recovered from fine particle samples (with and without mask) of 2/37 subjects.
- Virus culture not attempted for the coarse fraction.

Milton et al., PLoS Pathog. 2013;9(3):e1003205.

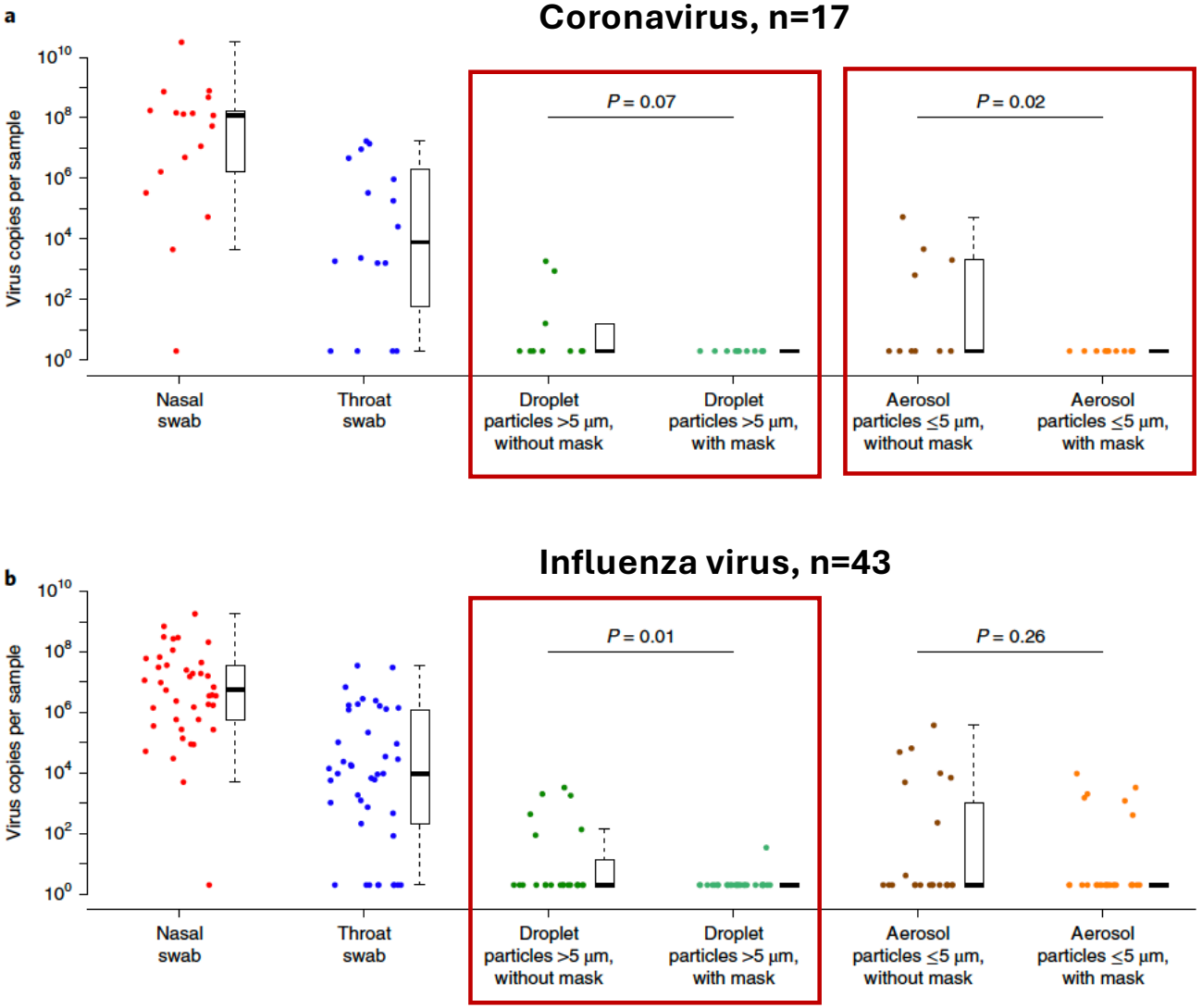
BRIEF COMMUNICATION
https://doi.org/10.1038/s41591-020-0843-2
nature medicine
Check for updates

Respiratory virus shedding in exhaled breath and efficacy of face masks

Nancy H. L. Leung[1], Daniel K. W. Chu[1], Eunice Y. C. Shiu[1], Kwok-Hung Chan[2], James J. McDevitt[3], Benien J. P. Hau[1,4], Hui-Ling Yen[1], Yuguo Li[5], Dennis K. M. Ip[1], J. S. Malik Peiris[1], Wing-Hong Seto[1,6], Gabriel M. Leung[1], Donald K. Milton[7,8] and Benjamin J. Cowling[1,8]



Leung et al. Nat Med. 2020;26(5):676-680.



Relative efficacy of masks and respirators as source control for viral aerosol shedding from people infected with SARS-CoV-2: a controlled human exhaled breath aerosol experimental study

Jianyu Lai,[a] Kristen K. Coleman,[a] S.-H. Sheldon Tai,[a] Jennifer German,[a] Filbert Hong,[a] Barbara Albert,[a] Yi Esparza,[a] Dewansh Rastogi,[b] Aditya Srikakulapu,[a] Petri Kalliomäki,[a] Maria Schanz,[a] Alycia A. Smith,[a] Isabel Sierra Maldonado,[a] Molly Oertel,[a] Naja Fadul,[a] T. Louie Gold,[a] Kathleen McPhaul,[a] Tianzhou Ma,[c] Benjamin J. Cowling,[d] and Donald K. Milton[a,*]



Clothe mask



Surgical mask



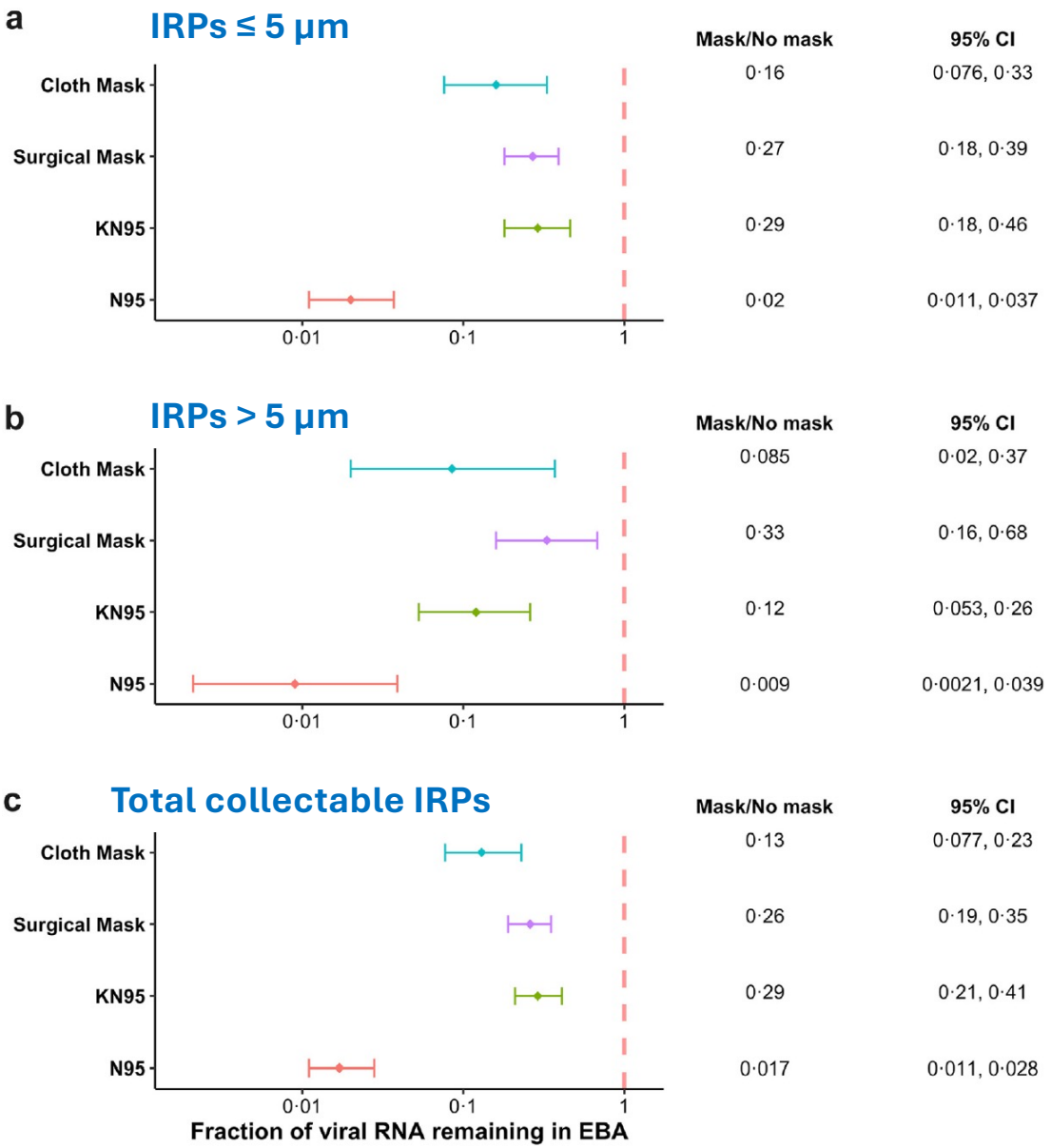
KN95



Duckbill N95

- Exhaled breath of 44 COVID-19 volunteers with paired unmasked and masked breath samples.
- All masks and respirators significantly reduced exhaled viral load.
- **Duckbill N95** reduced exhaled viral load by 98% (95% CI: 97–99%) and significantly outperformed KN95 (71%, 59–79%), surgical mask (74%, 65–81%) and clothe mask (87%, 77–92%).

PMID: 38821778



Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community

Jing Yan[a,b], Michael Grantham[a,1], Jovan Pantelic[a,2], P. Jacob Bueno de Mesquita[a], Barbara Albert[a], Fengjie Liu[a,3], Sheryl Ehrman[b,4], Donald K. Milton[a,5], and EMIT Consortium[6]

	NP swab		Coarse aerosol		Fine aerosol	
Parameter	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Age	0.85** (0.72–1.0)	0.78*** (0.66–0.92)	1.0 (0.90–1.2)	1.0 (0.90–1.1)	0.99 (0.88–1.1)	1.0 (0.90–1.1)
Male	0.34 (0.14–2.5)	0.68 (0.17–2.8)	1.7 (0.52–5.6)	1.7 (0.54–5.5)	2.4* (0.88–6.5)	1.3 (0.39–4.2)
Asthma	3.8 (0.63–22.9)	—	1.38 (0.32–6.04)	—	2.6 (0.77–8.95)	—
Smoker	2.3 (0.30–18.2)	—	0.48 (0.09–2.7)	—	1.5 (0.36–6.1)	—
BMI	1.3 (0.58–2.7)	—	1.9* (0.92–3.98)	1.8 (0.83–3.8)	1.7* (0.98–2.9)	2.5*** (1.4–4.3)
Vaccination† (current year)	1.3 (0.22–7.6)	—	1.4 (0.34–5.9)	—	2.8* (0.85–9.3)	—
Vaccination (previous year)	1.55 (0.45–5.5)	—	0.71 (0.15–3.3)	—	2.3 (0.79–6.6)	—
Vaccination (current and previous)	0.77 (0.10–5.7)	—	1.13 (0.22–5.7)	—	2.1*** (1.3–3.2)	6.3*** (1.9–21.5)
Antiviral medication	1.1 (0.03–33.9)	—	0.64 (0.04–11.5)	—	1.04 (0.09–12.0)	—
Influenza A	0.67 (0.13–3.3)	—	1.0 (0.18–5.5)	—	0.44 (0.13–1.5)	—
Log (NP swab)	—	—	1.3 (0.91–1.82)	—	1.2 (0.76–1.78)	—
Day 2 postonset	2.13 (0.48–9.5)	—	0.34* (0.10–1.02)	0.34* (0.10–1.1)	0.44* (0.18–1.05)	0.47* (0.20–1.1)
Day 3 postonset	0.55 (0.11–2.8)	—	0.29* (0.08–1.03)	0.30 (0.08–1.03)	0.20**** (0.08–0.52)	0.24*** (0.09–0.59)
Fever‡	3.6 (0.76–17.2)	—	0.70 (0.19–2.7)	—	1.7 (0.64–4.5)	—
No. of coughs	1.3 (0.65–2.8)	—	2.1*** (1.2–3.8)	2.3*** (1.3–4.0)	2.9***** (1.9–4.6)	2.6**** (1.6–4.2)
Upper respiratory symptoms	3.4**** (1.8–6.6)	2.9*** (1.5–5.7)	0.76 (0.44–1.3)	—	0.88 (0.58–1.4)	—
Lower respiratory symptoms	3.2* (0.96–10.8)	1.2 (0.32–4.8)	0.94 (0.36–2.5)	—	1.9 (0.86–4.1)	—
Systemic symptoms	5.3*** (1.6–17.7)	2.4 (0.31–18.3)	0.86 (0.33–2.3)	—	1.8 (0.82–3.7)	—
Male × no. of coughs	—	—	—	—	—	3.2** (1.1–9.3)

- Young adults (n=335) with respiratory infection were recruited in 2012-2013.
- 142 cases with confirmed influenza infection and provided paired nasopharyngeal (NP) and breath samples.
- **Viral load in NP swabs was not a significant predictor of aerosol shedding.**
- Fine- and coarse-aerosol viral RNA were positively associated with BMI and number of coughs, but negatively associated with increasing days since symptom onset.

PMID: 29348203

Impact of Ventilation on Respiratory Virus Transmission in College Residence Hall Cohorts: Potential for Causal Inference About Mode of Transmission

T. Louis Gold[1] | Kathleen M. McPhaul[1] | Huang Lin[2] | Ryan Doughty[3] | Irina Maljkovic Berry[4] | Filbert Hong[1] | Jianyu Lai[1] | Todd J. Treangen[3,5] | Jelena Srebric[6] | Donald K. Milton[1]

TABLE 3 | ARI within roommate pairs in high- and low-ventilation buildings by year.

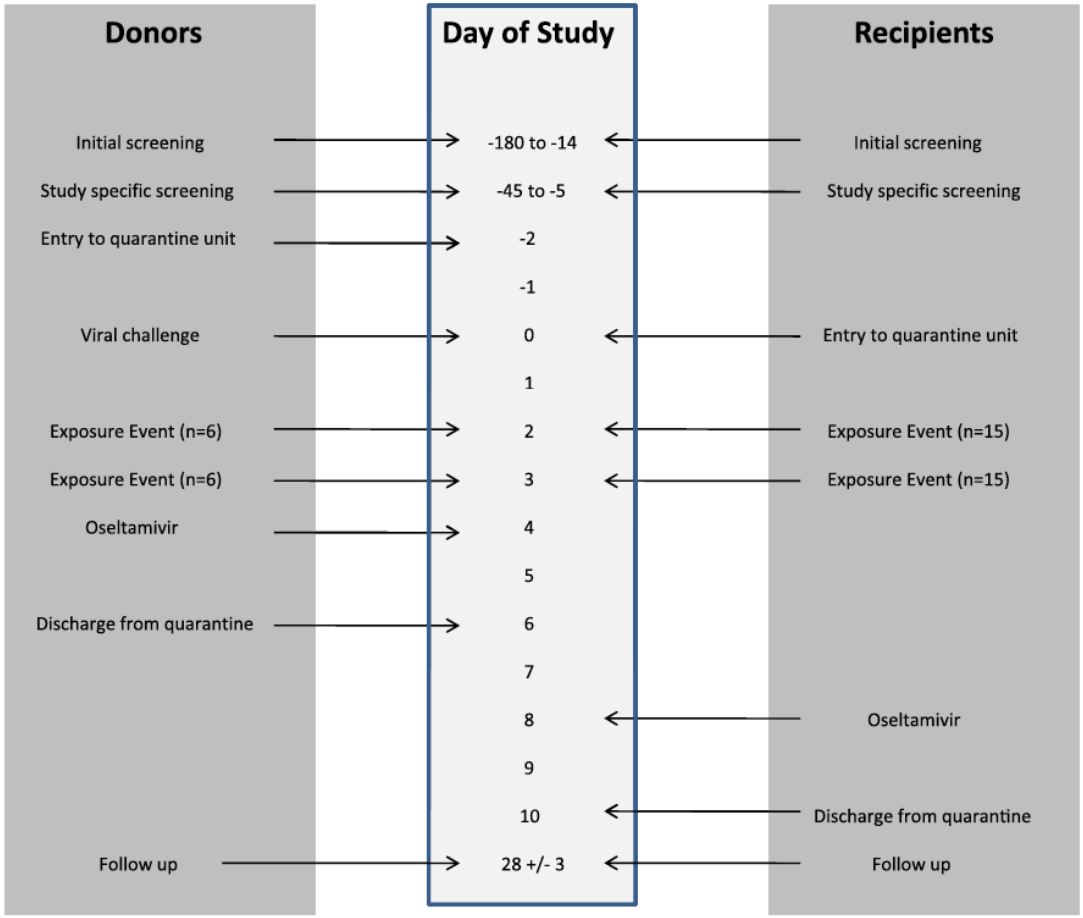
		Year 1		Year 2	
Residence hall ventilation	**Overall**	**HVent**	**LVent**	**HVent**	**LVent**
Enrolled roommate pairs[a]	97	2	43	12	40
Transmission cohort[b]	39	0	18	2	19
Pair-surveillance intervals[c]	64	0	26	3	35
Transmission-risk intervals[d]	36	0	18	2	16
Possible transmission[e]	4	0	1	0	3
Probable transmission cluster[f]	2	0	1	0	1
Probable transmission[g]	1	0	0	0	1
Possible transmission risk[h]	11.1%	0.0%	5.6%	0.0%	18.8%
Possible transmission count[i]					
CoVs	3	0	0	0	3
IBV	1	0	1	0	0
RSVA or RSVB	0	0	0	0	0

PMID: 40604338

- **High ventilation hall (≥ 5 L/s per person) vs. Low ventilation hall (< 5 L/s per person).**
- Prospective cohort recruited in 2018 and 2019, focus on 97 recruited roommate pairs with concordant PCR results tested within 2-weeks.
- 36 roommate pairs had at least one pair member tested a positive PCR test (eg. transmission-risk intervals). These include 2 pairs living in HVent and 34 pairs living in LVent halls.
- **Transmission was detected in 4/34 LVent halls, and in 0/2 HVent halls.**
- Sample size estimation: 255 roommate pairs needed for each building to detect 50% reduction in transmission (power=80%, p=0.05)

Use of a Human Influenza Challenge Model to Assess Person-to-Person Transmission: Proof-of-Concept Study

Ben Killingley,[1] Joanne E. Enstone,[1] Jane Greatorex,[2] Anthony S. Gilbert,[3] Rob Lambkin-Williams,[3] Simon Cauchemez,[4] Jacqueline M. Katz,[5] Robert Booy,[6] Andrew Hayward,[7] John Oxford,[8] Carolyn B. Bridges,[5] Neil M. Ferguson,[4] and Jonathan S. Nguyen Van-Tam[1]



- 9 donors were inoculated with **$10^{5.5}$ $TCID_{50}$ of A/Wisconsin/67/2005 (H3N2).**
  - 7/9 donors were found to be infected (PCR positive plus seroconversion).
  - Among the 7 infected donors, 4 were symptomatic and 3 were asymptomatic.
- 15 contacts were exposed to donors on day 2 and 3 inoculation in three groups (2 donors plus 5 contacts).
- Laboratory confirmed case: seroconversion by ≥4 fold, or positive test by PCR or culture.

PMID: 22131338

30 hour (3 x 10 hours) exposure events on day 2 and day 3 post-inoculation



Group A Subjects	Symptoms post virus exposure	ILI	Fever	Influenza status
D03	Yes	Yes	No	Infected
D04	No	No	No	Immune
R01	No	No	No	Immune
R02	Yes	No	No	Not Infected
R03	Yes	No	No	Not Infected
R04	Yes	Yes	No	Not Infected[a]
R05	Yes	No	No	Not Infected
Attack rate = 0%				

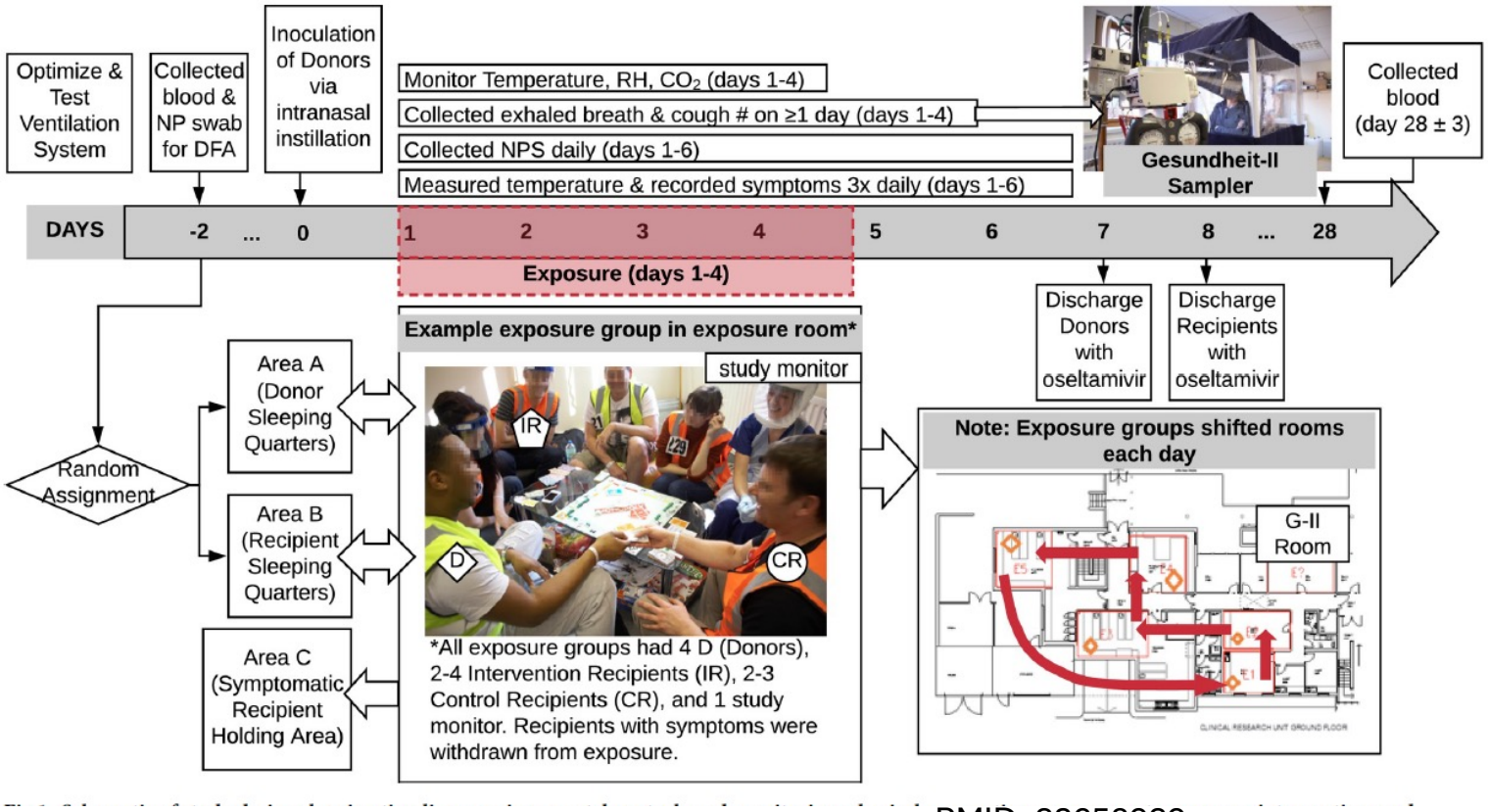
Group B Subjects	Symptoms post virus exposure	ILI	Fever	Influenza status
D01	Yes	Yes	No	Infected
D08[b]	No	No	No	Infected
D05[c]	Yes	Yes	Yes	Infected
R06	No	No	No	Not Infected
R07	Yes	Yes	No	Immune
R08	Yes	Yes	No	Infected
R09	No	No	No	Not Infected
R10	Yes	No	No	Not Infected
Attack rate = 20% (adjusted for immune Recipients = 25%)				

Group C Subjects	Symptoms post virus exposure	ILI	Fever	Influenza status
D02	Yes	Yes	No	Infected
D06	Yes	No	No	Not Infected
R11	Yes	No	No	Not Infected
R12	No	No	No	Infected
R13	Yes	No	No	Immune
R14	No	No	No	Not Infected
R15	Yes	Yes	No	Infected[a]
Attack rate = 40% (adjusted for immune Recipients = 50%)				

- The overall attack rate was 20% (3/15), since 3/15 contacts were found to be immuned, the adjusted attack rate was 25% (3/12).
  - Among the 3 infected recipients, 2 were symptomatic and 1 was asymptomatic.
- Influenza virus was detected by PCR from surface swabs (9/48, 19%) collected on day 2 and day 3.
- Air sampling detected 1/18 NIOSH sampler positive for influenza virus by PCR.

Minimal transmission in an influenza A (H3N2) human challenge-transmission model within a controlled exposure environment

Jonathan S. Nguyen-Van-Tam[1], Ben Killingley[1¤a]*, Joanne Enstone[1], Michael Hewitt[1], Jovan Pantelic[2¤b], Michael L. Grantham[2¤c], P. Jacob Bueno de Mesquita[2], Robert Lambkin-Williams[3], Anthony Gilbert[3], Alexander Mann[3], John Forni[3¤d], Catherine J. Noakes[4], Min Z. Levine[5], LaShondra Berman[5], Stephen Lindstrom[5], Simon Cauchemez[6¤e], Werner Bischoff[7], Raymond Tellier[8], Donald K. Milton[2], for the EMIT Consortium[¶]



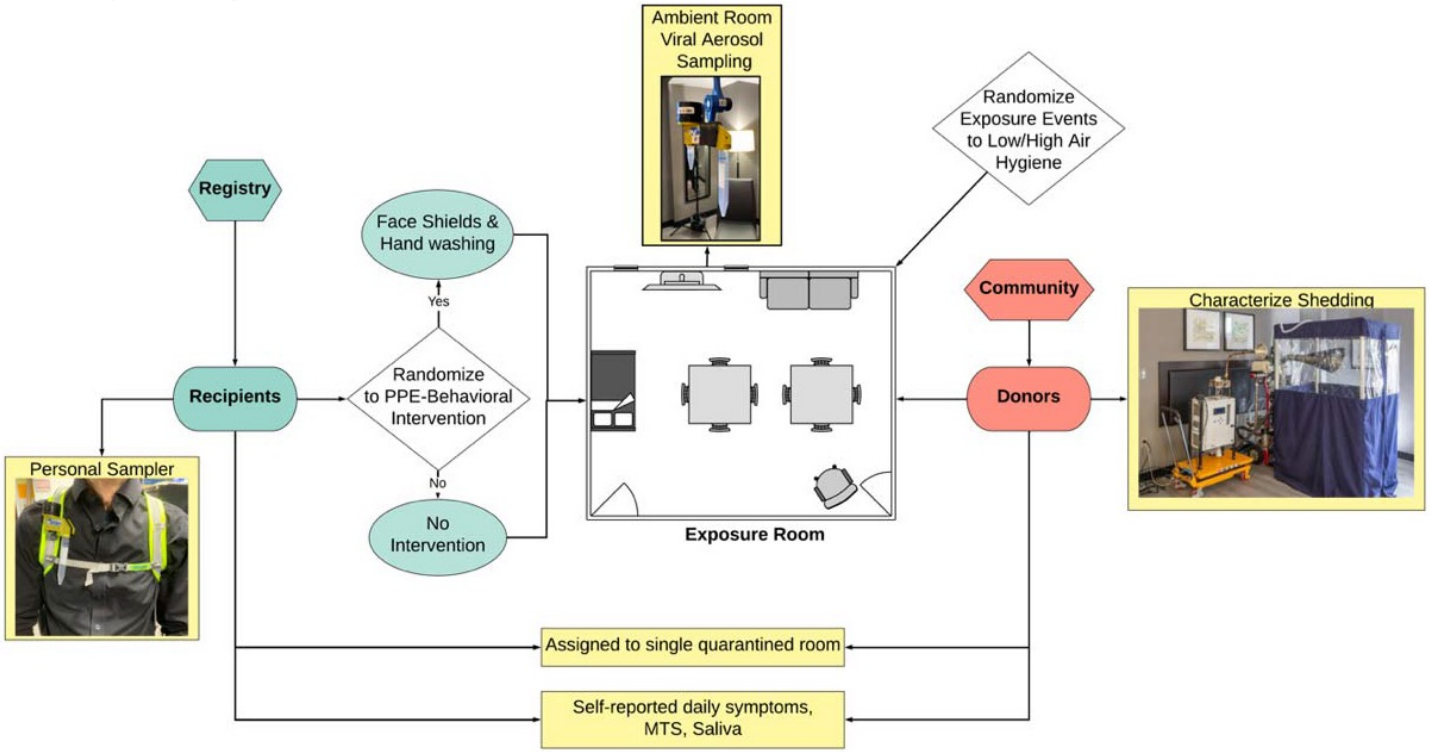
PMID: 32658939

- **Donors (n = 52)** were challenge with 10^5 $TCID_{50}$ of (H3N2).
- Recipients were randomized to **Intervention (IR, n = 40)** or **Control (CR, n = 35)**, and were exposed to Donors for **4 days**.
- **IRs wore face shields and hand sanitized frequently**.
- **One transmission was confirmed by serology** in the CR, yielding a secondary attack rate of 2.9% among CR, 0% in IR.
- Transmission was significantly less than the pilot study. The later study has better **mechanical building ventilation**, suggesting a possible role for aerosols.
- In addition, **donors had minimal shedding of virus (compared to community acquired infections).**

Evaluating Modes of Influenza Transmission (EMIT-2): Challenges of a Controlled Human Influenza Virus Infection Transmission Trial (CHIVITT)

Jianyu Lai, Hamed Sobhani, Kristen K. Coleman, S.-H. Sheldon Tai, Filbert Hong, Isabel Sierra Maldonado, Yi Esparza, Kathleen M. McPhaul, Shengwei Zhu, Don L. DeVoe, Justin R. Ortiz, Shuo Chen, Temima Yellin, Juan Manuel Carreno, Florian Krammer, Benjamin J. Cowling, Aubree Gordon, Wilbur H. Chen, Jelena Srebric, Donald K. Milton, the EMIT-2 Study Team

doi: https://doi.org/10.1101/2025.04.28.25326458

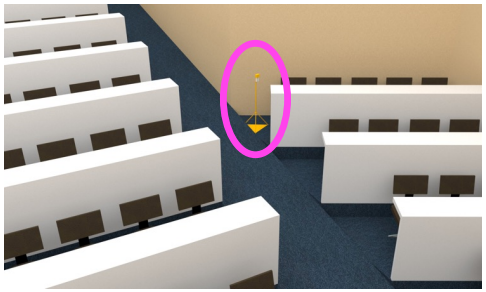


- **Community recruited recipients and donors (naturally acquired infection, within 48h symptom onset).**
- **5 donors and 11 recipients were recruited in 2024**.
  - 24b: Donor =1, IR=3, CR=5, exposure= 6 events (2-4h)
  - 24c: Donor = 4, IR=1, CR=2, exposure=17 events
- Exposure conducted in a quarantine hotel with very low ventilation (0.25-0.5 ACH). No transmission was observed.
- Influenza viral RNA was detected in donor exhaled breath, but at lower levels than subjects recruited pre-COVID.

https://www.medrxiv.org/content/10.1101/2025.04.28.25326458v1.full.pdf+html

Air sampling at a university campus

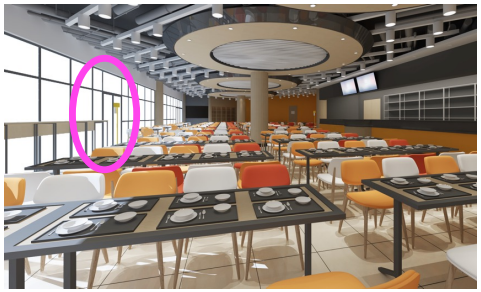
Lecture halls



University Health Service



Canteens

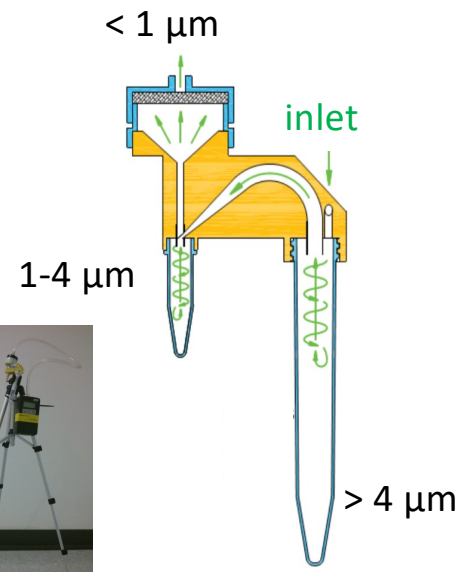


Shuttle buses



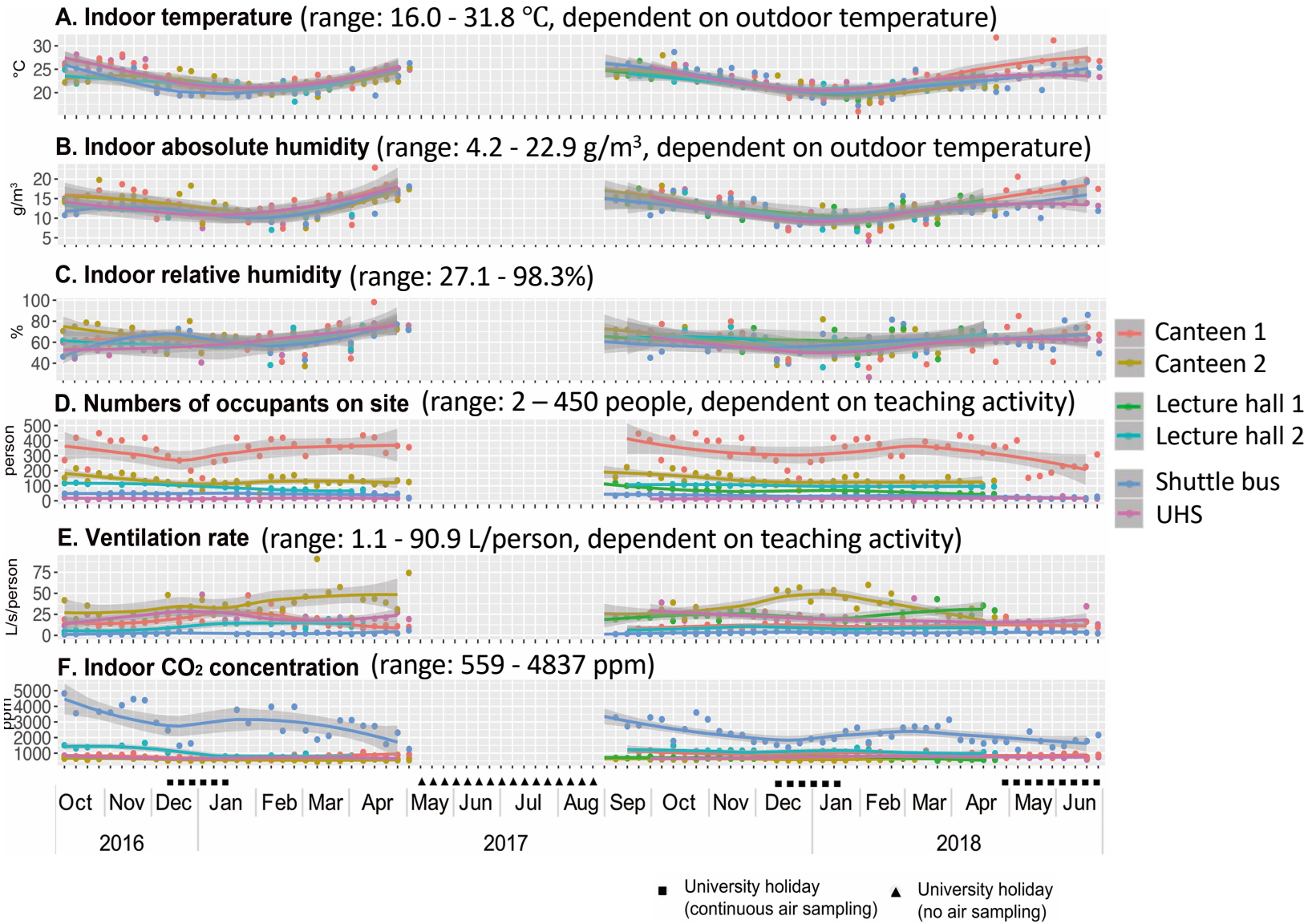
NIOSH bioaerosol sampler





- From **October 2016 to June 2018**, air was sampled weekly using 6-10 samplers per week except during summer holidays in 2017. **There were a total 334 sampling events with 1028 air samples collected.**
- **Environmental factors** (indoor/ outdoor temperature and relative humidity levels, indoor CO_2 concentrations) and **ecological factors** (number of occupants on site) were recorded during each sampling event.

PMID: 30963180


A. Indoor temperature (range: 16.0 - 31.8 ℃, dependent on outdoor temperature)
°C
30
25
20
B. Indoor abosolute humidity (range: 4.2 - 22.9 g/m³, dependent on outdoor temperature)
g/m³
20
15
10
5
C. Indoor relative humidity (range: 27.1 - 98.3%)
%
100
80
60
40
D. Numbers of occupants on site (range: 2 – 450 people, dependent on teaching activity)
person
500
400
300
200
100
0
E. Ventilation rate (range: 1.1 - 90.9 L/person, dependent on teaching activity)
L/s/person
75
50
25
0
F. Indoor CO₂ concentration (range: 559 - 4837 ppm)
ppm
5000
4000
3000
2000
1000
Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun
2016
2017
2018
Canteen 1
Canteen 2
Lecture hall 1
Lecture hall 2
Shuttle bus
UHS
University holiday (continuous air sampling)
University holiday (no air sampling)

Detection of influenza virus & other respiratory viruses in air sampled from university campus

	Positive rate (%)	
	Viral RNA detected by RT-PCR (positive samples/ air samples collected)	**Infectious virus detected *in vitro*** (positive samples/ RT-PCR positive samples)
Influenza viruses		
Influenza A virus (IAV)	**16.9% (117/694)**	5% (6/109)
Influenza B virus (IBV)	**4.5% (31/694)**	3% (1/31)
Both influenza A and B viruses	0.7% (5/694)	N.D.
Other respiratory viruses		
Human rhinoviruses (HRV)	2.2% (6/270)	0% (0/6)
Respiratory syncytial viruses (RSV)	0.4% (1/270)	0% (0/1)
Human coronaviruses (HCoV 229E/ OC43)	0% (0/152)	0% (0/0)

N.D., Not determined.
Majority (75.2%, 88/117) of IAV positive samples were not subtypable due to low copy of viral genome sampled in air. H1 (6.8%, 8/117), H3 (16.2% 19/117), and H1 plus H3 (1.7%, 2/117).

Crowdedness and indoor temperature were associated with the detection of influenza viral RNA in air

Table 3. Multivariable Analyses on Factors Associated With the Detection of Influenza Viral RNA in Air

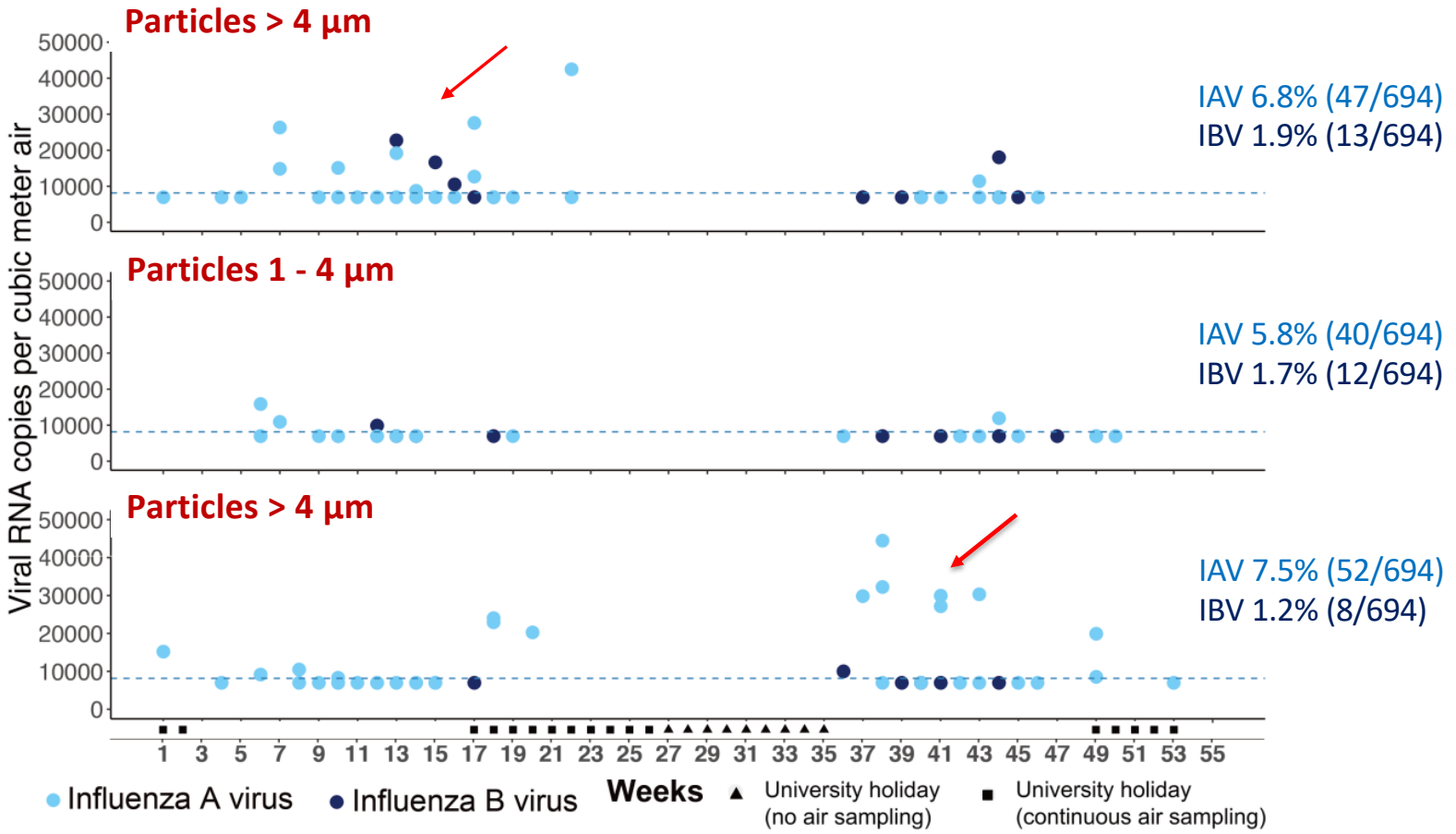
	Best Fit Model	Competing Model 1	Competing Model 2	Competing Model 3
Risk Factor	aOR (95% CI); *P* Value	aOR (95% CI); *P* Value	aOR (95% CI); *P* Value	aOR (95% CI); *P* Value
Crowdedness (No. of occupants on site)[a]				
Low	Reference	Reference	Reference	Reference
Intermediate	1.21 (.73–2.04); $P = .461$	1.22 (.73–2.05); $P = .452$	1.18 (.70–2.00); $P = .530$	1.16 (.69–1.95); $P = .585$
High	2.29 (1.44–3.71); $P < .001$	2.33 (1.46–3.79); $P < .001$	2.19 (1.36–3.60); $P = .002$	2.13 (1.33–3.49); $P = .002$
Indoor temperature (°C)				
Numeric	1.14 (1.05–1.23); $P = .001$	1.19 (1.07–1.33); $P = .001$	1.12 (1.02–1.23); $P = .014$	1.13 (1.04–1.23); $P = .003$
Indoor AH (kg/m^3)				
Numeric (per 0.002 increase)	…	0.89 (.74–1.07); $P = .213$	…	…
Outdoor AH (kg/m^3)				
Numeric (per 0.002 increase)	…	…	1.03 (.95–1.12); $P = .475$	…
AH difference (indoor–outdoor) (kg/m^3)				
Numeric (per 0.002 increase)	…	…	…	0.94 (.86–1.03); $P = .181$

Sampling performed on teaching days vs holidays was highly associated with numbers of occupants on site (χ^2 test, $P < .001$); as such, crowdedness (number of occupants on site) was included in the multivariable analysis.

Abbreviations: AH, absolute humidity; aOR, adjusted odds ratio; CI, confidence interval.

[a]Number of occupants was categorized into high, intermediate, and low based on the numeric data distribution: high (upper 33%), intermediate (middle 33%), and low (lower 33%)

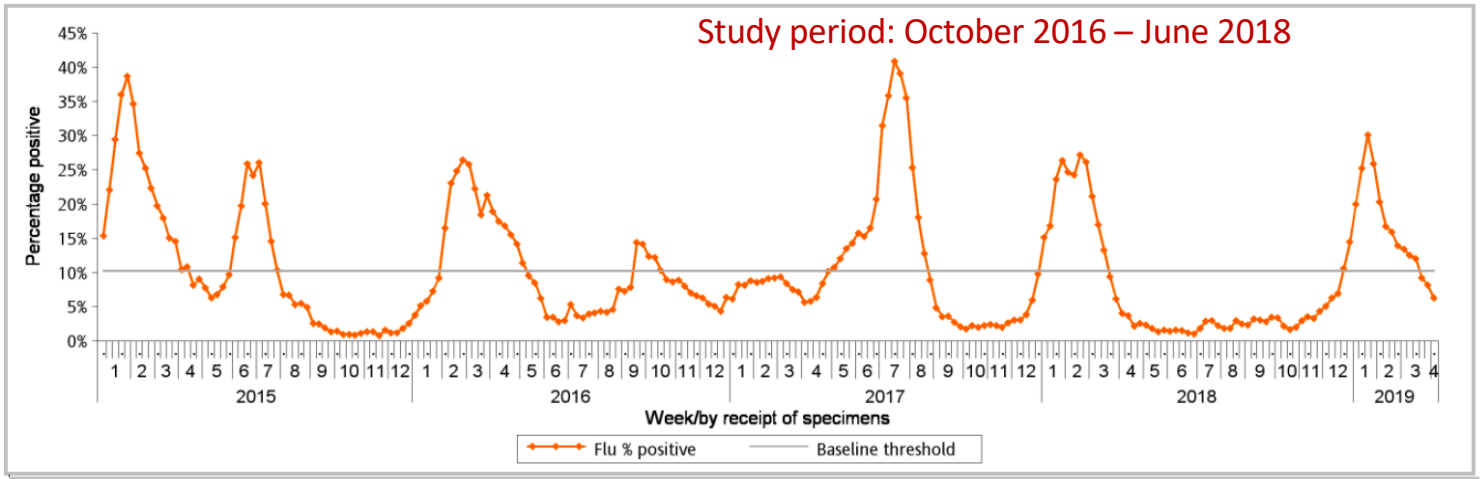
Higher copies of influenza viral RNA were detected from airborne particles > 4 µm in spring and < 1 µm in autumn

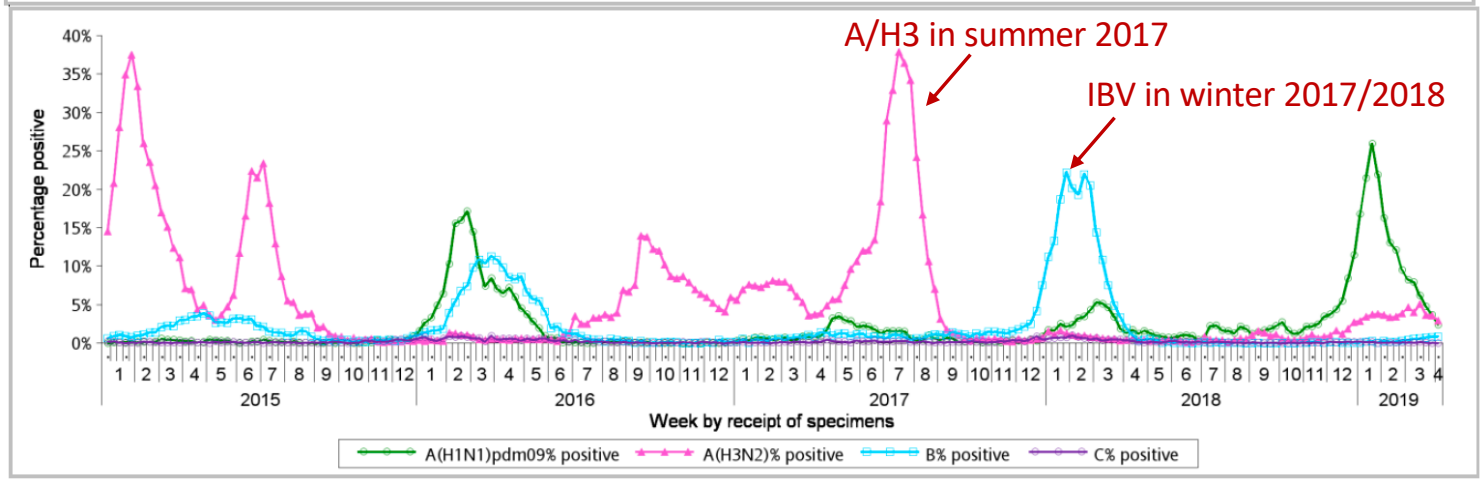


FLU EXPRESS

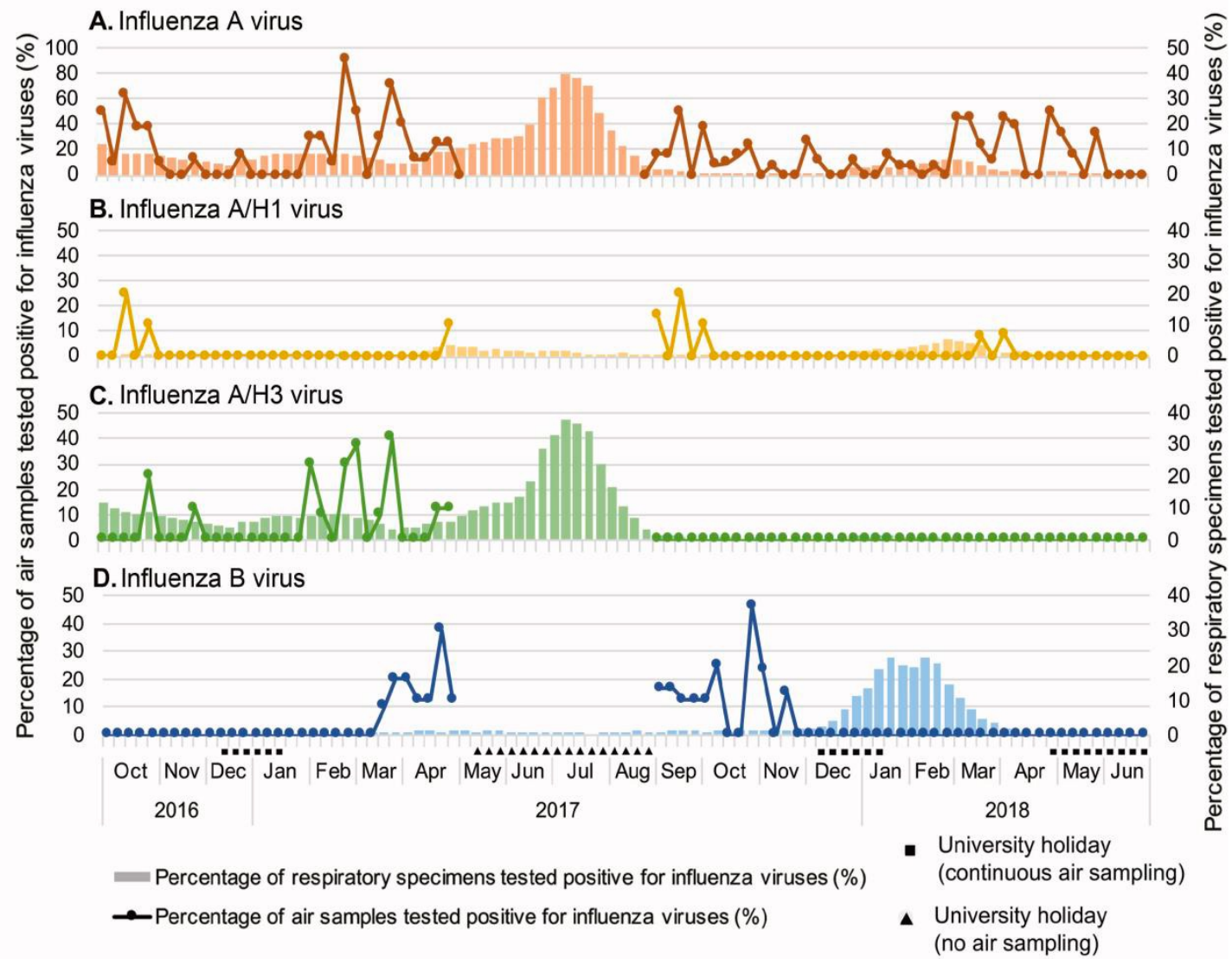
衞生防護中心
Centre for Health Protection
保障市民健康
Protecting Hong Kong's health

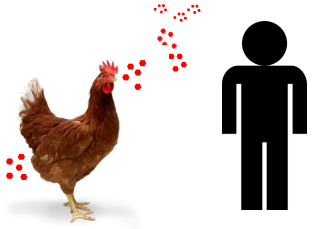
Flu Express *is a weekly report produced by the Respiratory Disease Office of the Centre for Health Protection. It monitors and summarizes the latest local and global influenza activities.*





Detection of A/H3 and influenza B viruses in air prior to the epidemics





Influenza transmission at human-animal interface

Transmission of H7N9 Influenza Viruses with a Polymorphism at PB2 Residue 627 in Chickens and Ferrets

Geraldine S. M. Luk,[a] Connie Y. H. Leung,[b] Sin Fun Sia,[b] Ka-Tim Choy,[b] Jie Zhou,[b] Candy C. K. Ho,[b] Peter P. H. Cheung,[b] Elaine F. Lee,[a] Chris K. L. Wai,[a] Pamela C. H. Li,[a] Sin-Ming Ip,[a] Leo L. M. Poon,[b] William G. Lindsley,[c] Malik Peiris,[b] Hui-Ling Yen[b]

Agriculture, Fisheries and Conservation Department, The Government of Hong Kong SAR, Hong Kong[a]; School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong[b]; Allergy and Clinical Immunology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA[c]

Mammalian adaptation marker

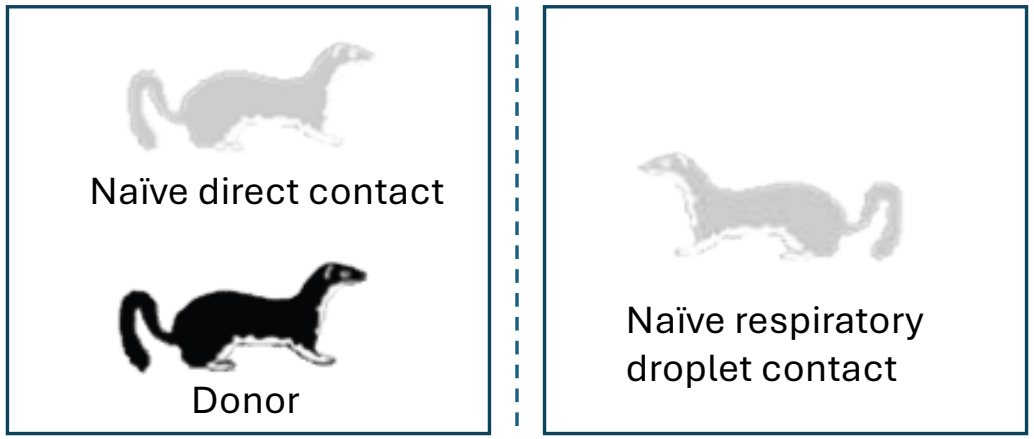
Viruses	PB2	PB1	PA	NP
Silkie chicken A/SCk/HK/1772/2014	E627	M372	S648	G485
Human A/HK/3263/2014	V/K/E 627	L372	N648	R485

V (60%); K (20%); E (20%)
Clonal sequencing

Genetically-related human and chicken H7N9 isolates were compared for their transmission potential:
- from ferrets to ferrets
- from chickens to chickens
- from chickens to ferrets

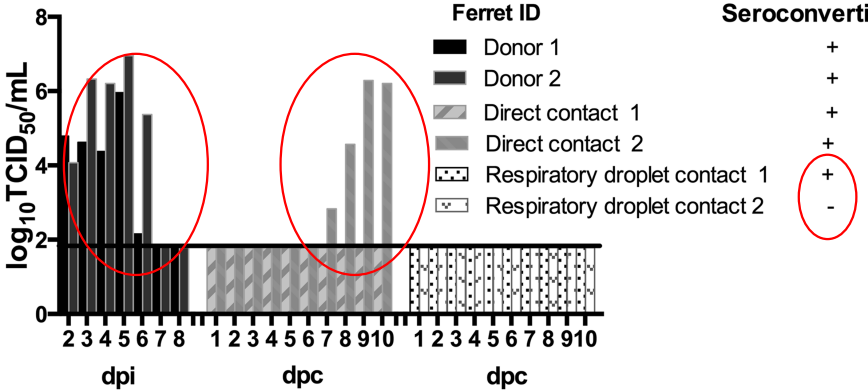
PMID: 26202239

Ferret to ferret transmission

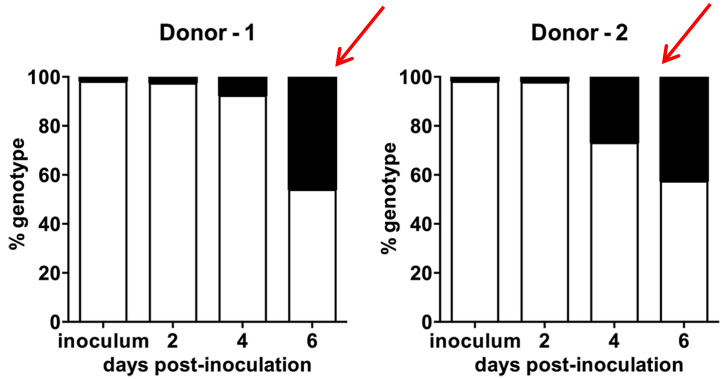


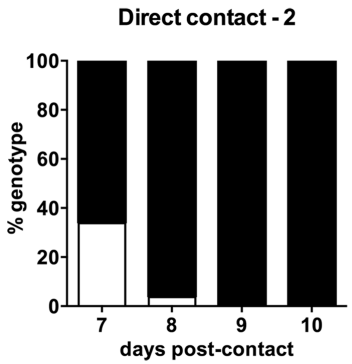
One of the parameters considered in the risk assessment tools, IRAT and TIPRA.

Ferret-to-ferret transmission –SCk1772



Positive selection for PB2-K627

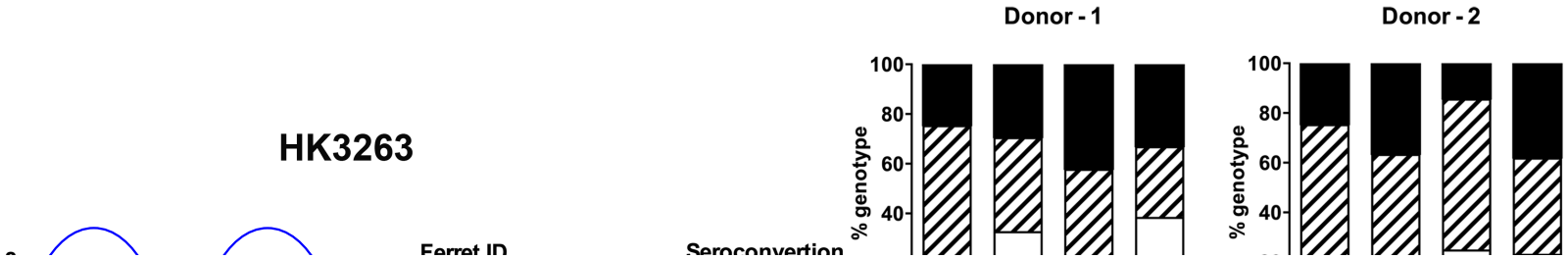




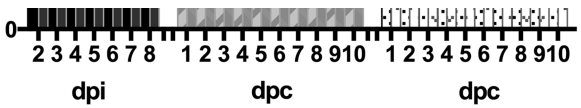
Transmission of both PB2-627K/E genotypes followed by rapid selection of K627.

627K
627E

Ferret-to-ferret transmission –HK3263

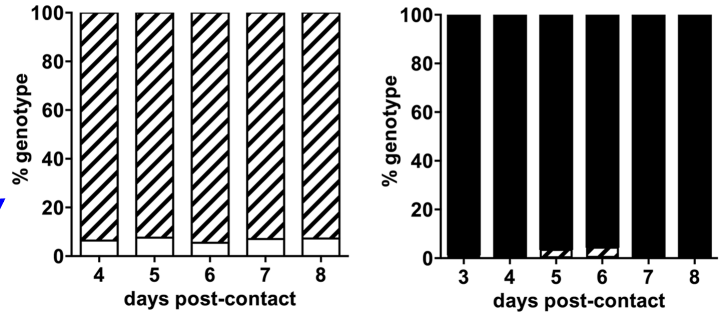


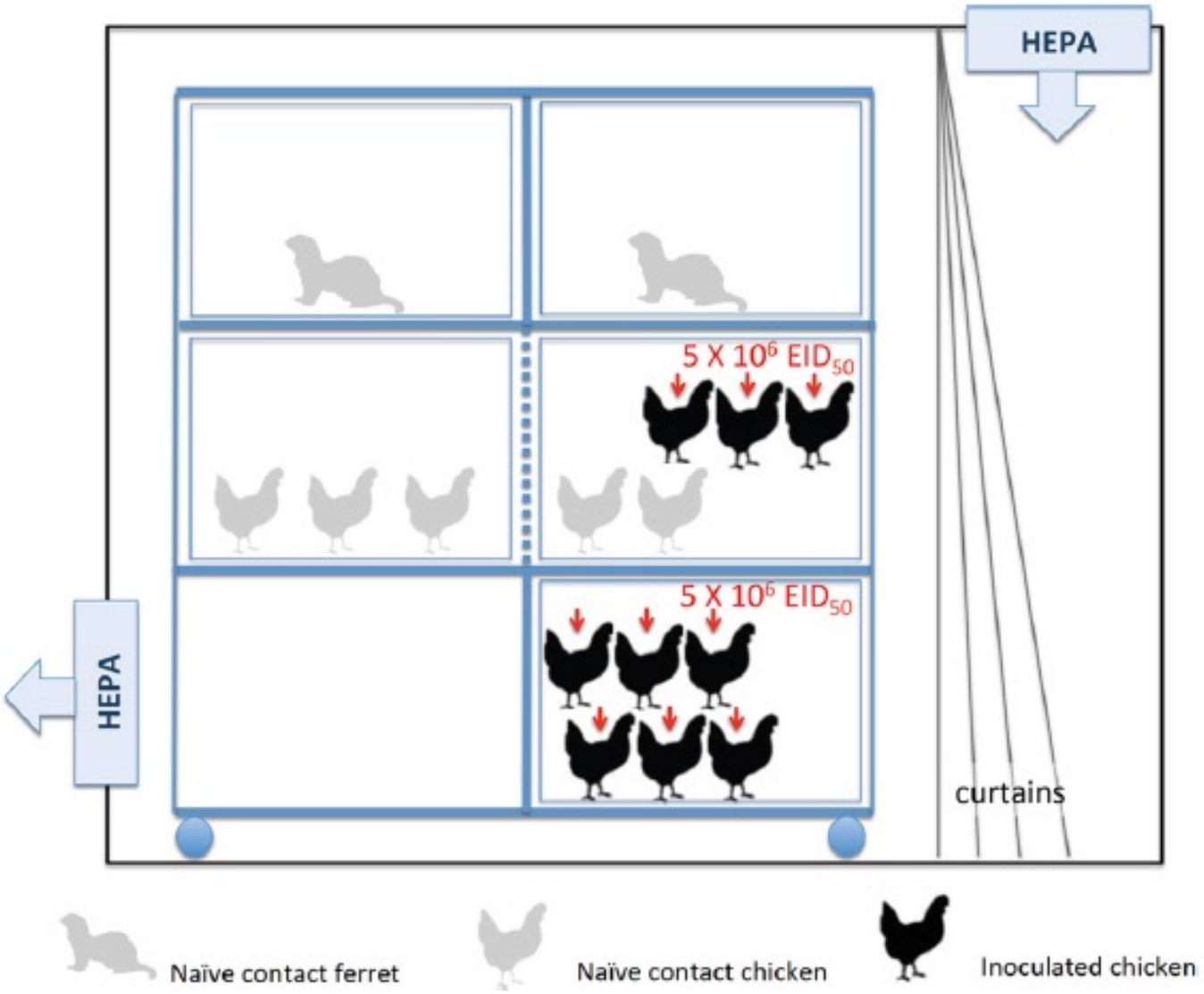
Ferret to ferret transmission via direct contact only



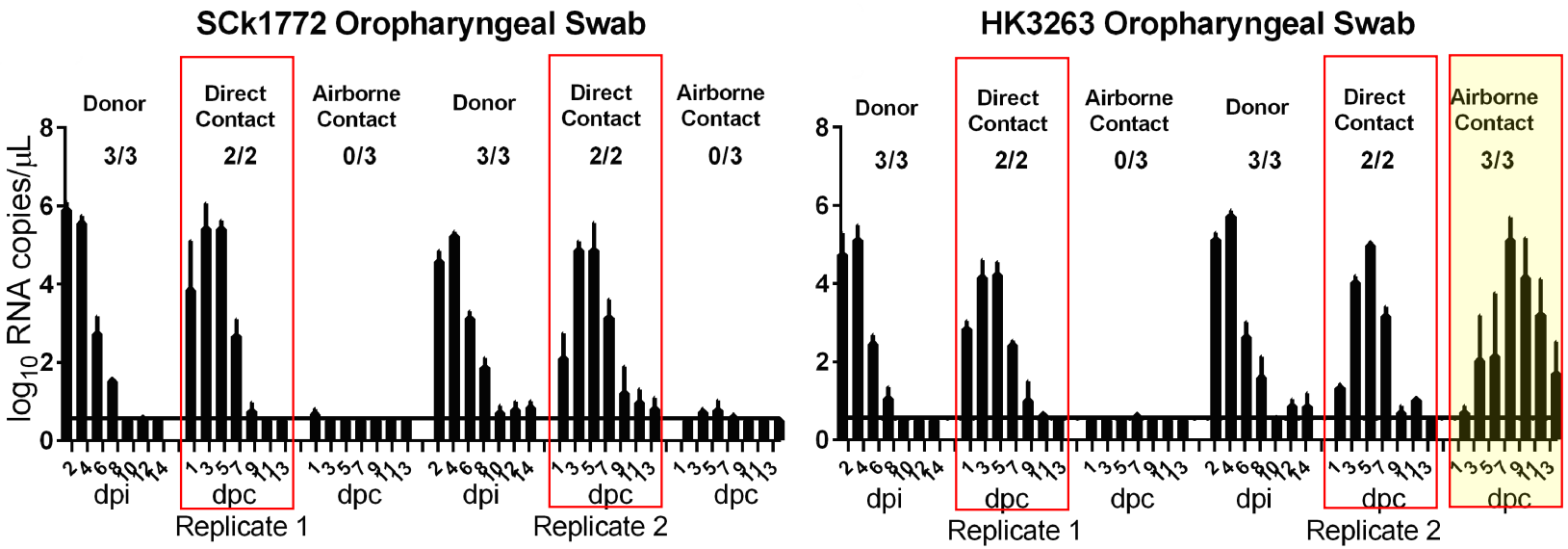
627K
627V
627E

Only PB2-627V/K were transmitted.



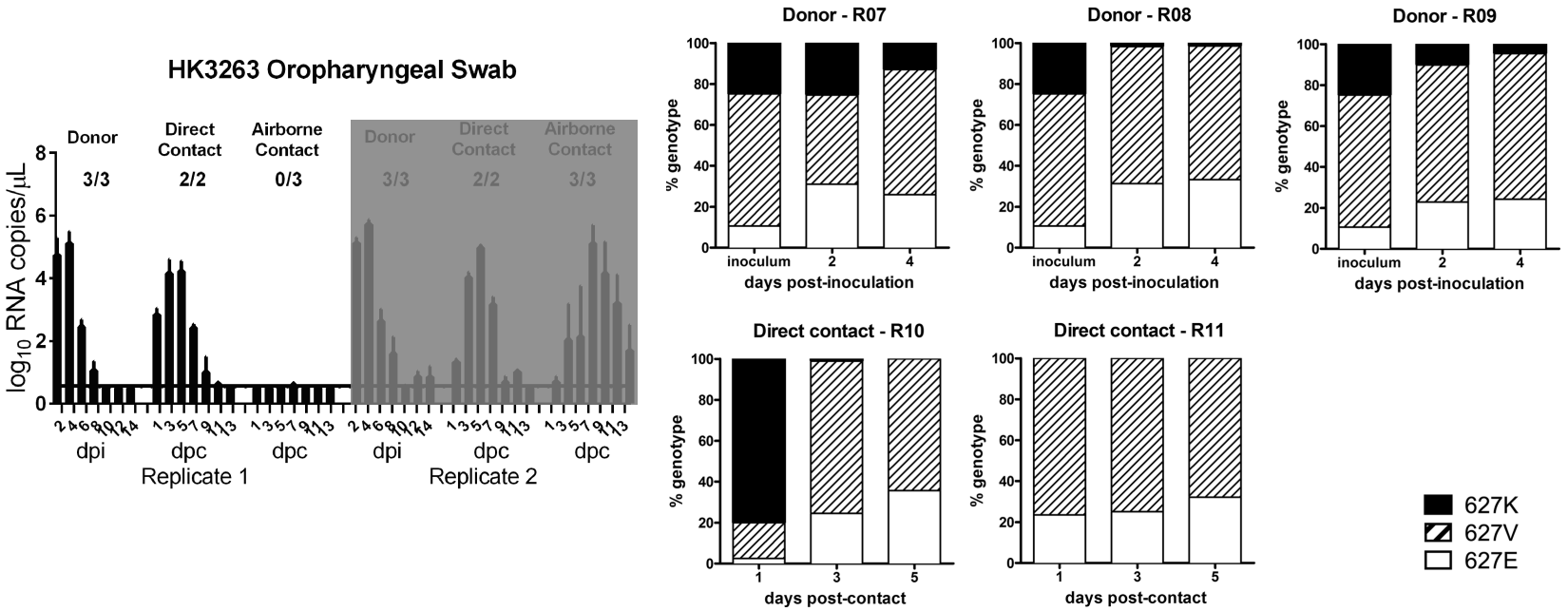

HEPA
5 X 10⁶ EID₅₀
5 X 10⁶ EID₅₀
HEPA
curtains
Naïve contact ferret
Naïve contact chicken
Inoculated chicken

H7N9 viruses transmit efficiently among chickens via direct contact

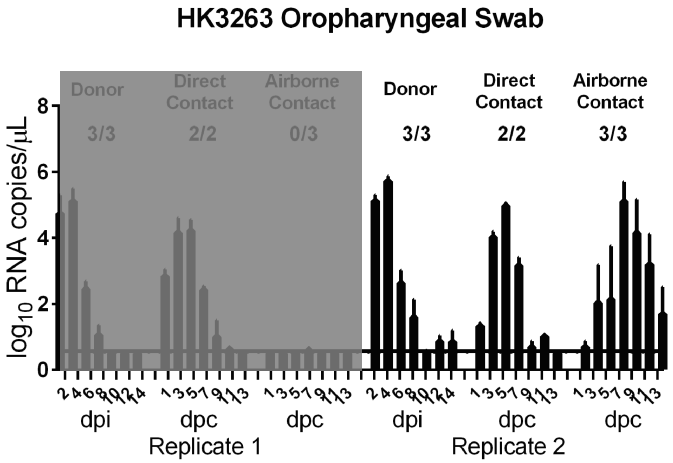


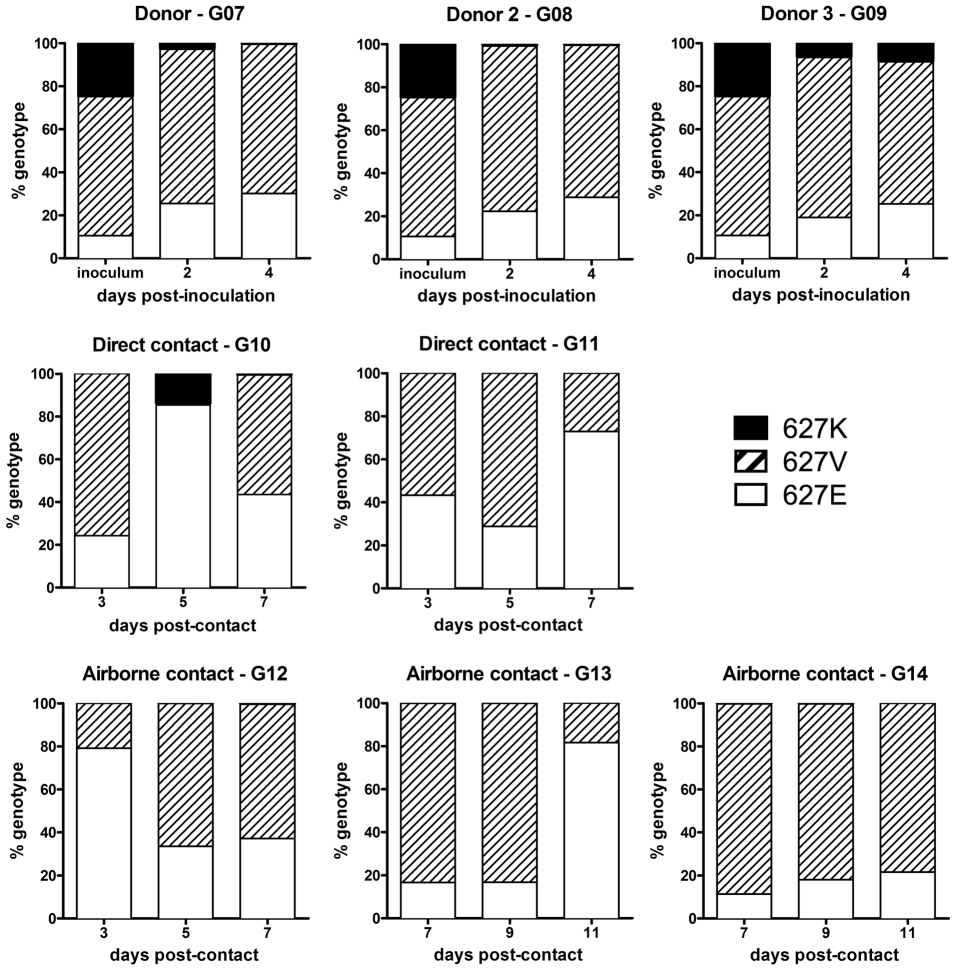
- Human and poultry H7N9 viruses showed comparable transmissibility.
  - Transmissions to direct contact (4/4) were detected by 24h post-exposure.
  - Transmission via airborne route was noted in 1/3 chicken on 3dpc (4.3 $\log_{10}$ RNA copies/µL) and from the other 2 cage mates on 7 dpc (5.5 -5.9 $\log_{10}$ RNA copies/µL).

PB2-627E/V were preferably transmitted among chickens

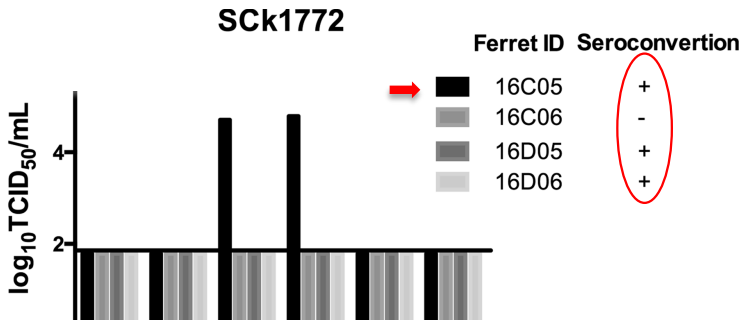


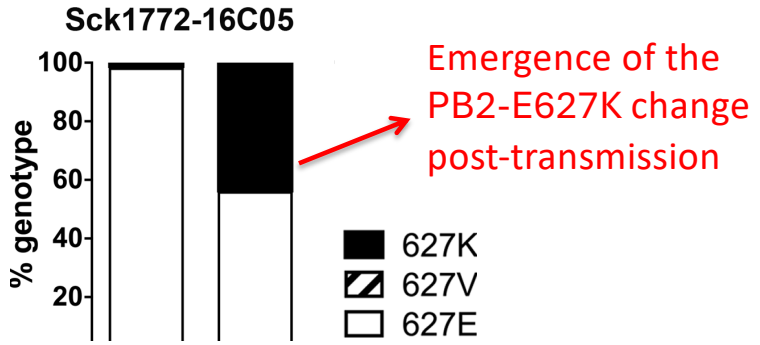
PB2-627E/V were preferably transmitted among chickens



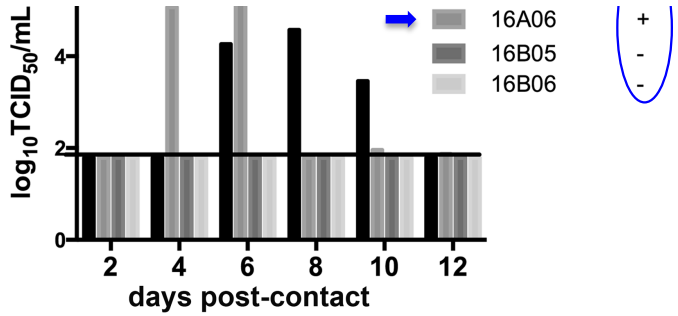


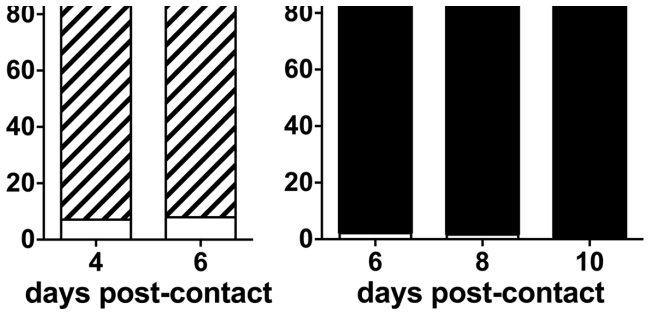
Chicken-to-ferret transmission via airborne route





Transmission via IRPs from chickens to ferrets is possible!





A narrow transmission bottle neck was observed.

Influenza viral RNA can be detected in the air



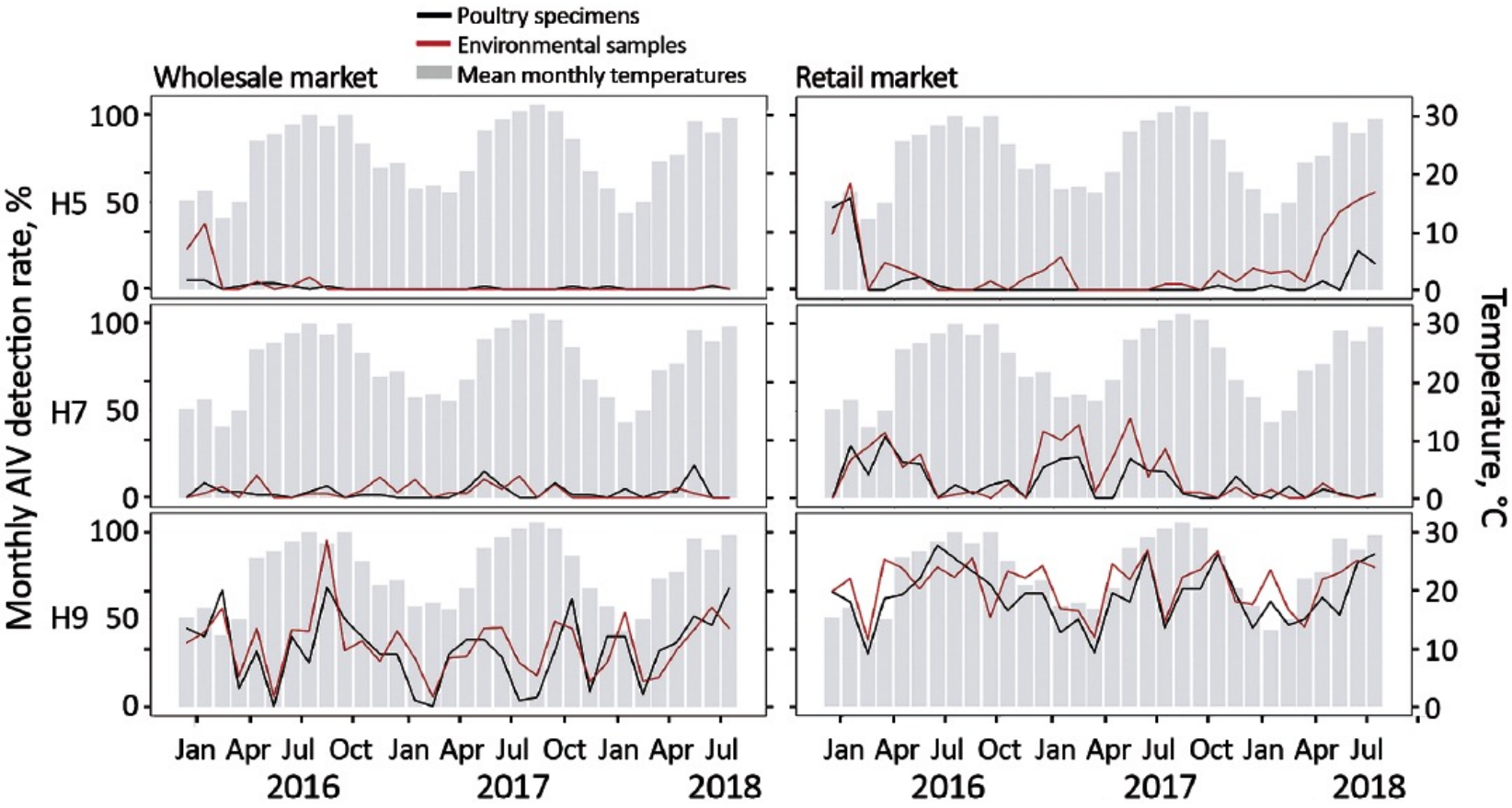
Air sampling (30 minutes at 3.5 liter air per minute) was performed during chicken sampling. Viral M gene copy numbers were determined by real-time quantitative PCR with the linear range limit of quantification at 3.71 copies per liter air at Ct =38.20.

Table 2. Detection of influenza M gene in the air using the NIOSH air sampler

		Detection of influenza M gene (Ct values)					
		SCk1772			HK3263		
		>4 µm	1-4 µm	<1 µm	>4 µm	1-4 µm	<1 µm
2 dpi	Replicate 1	-	-	-	-	-	-
	Replicate 2	-	-	-	+ (42.43)	-	-
4 dpi	Replicate 1	+ (41.12)	-	-	+ (40.80)	-	+ (44.51)
	Replicate 2	+ (44.23)	-	-	+ (40.57)	+ (40.91)	-
6 dpi	Replicate 1	+ (39.15)	-	-	+ (39.27)	-	-
	Replicate 2	+ (39.13)	-	-	+ (39.28)	-	-
8 dpi	Replicate 1	+ (39.77)	-	-	-	-	-
	Replicate 2	+ (37.46)	+ (36.76)	+ (38.97)	+ (37.28)	-	+ (38.58)

Avian Influenza Virus Detection Rates in Poultry and Environment at Live Poultry Markets, Guangdong, China

Kit Ling Cheng,[1] Jie Wu,[1] Wei Ling Shen, Alvina Y.L. Wong, Qianfang Guo, Jianxiang Yu, Xue Zhuang, Wen Su, Tie Song, Malik Peiris, Hui-Ling Yen,[2] Eric H.Y. Lau[2]



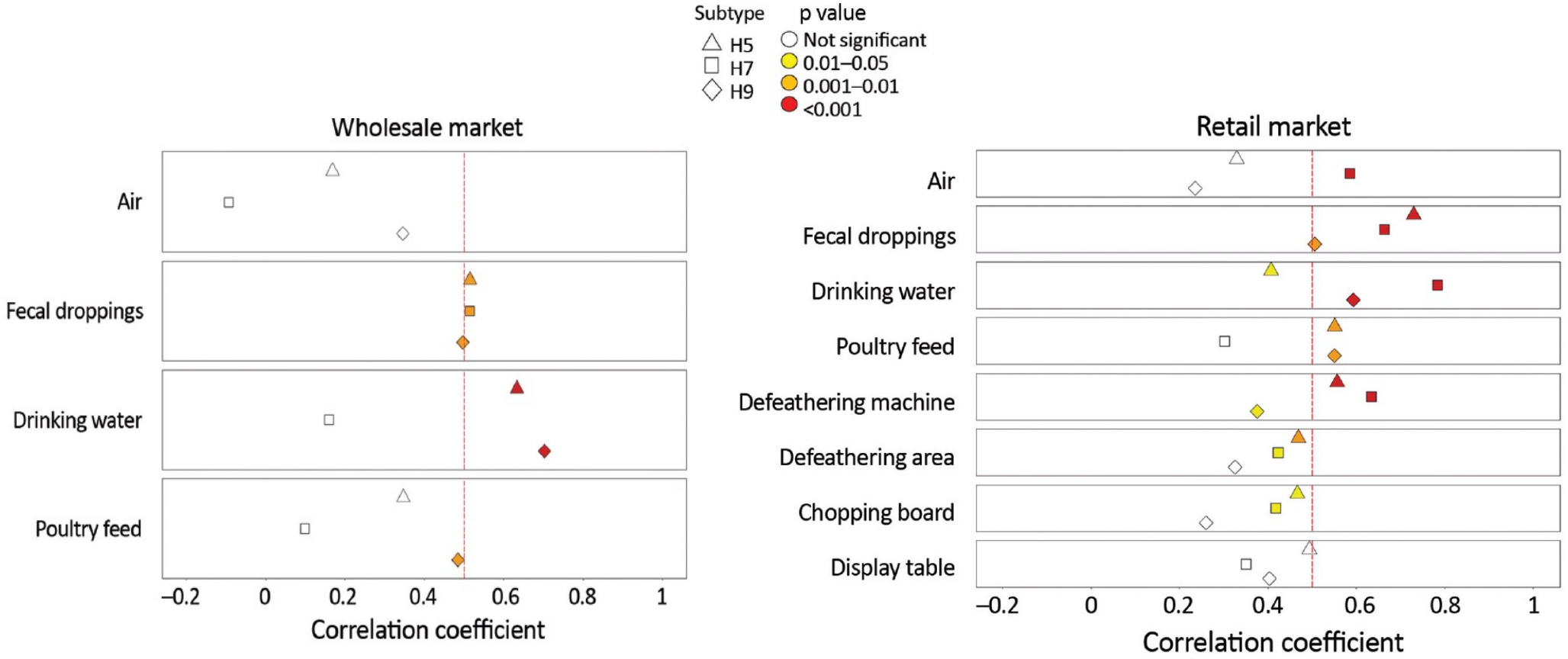
PMID: 31922954

Table 2. AIV detection rates from chicken and environmental samples collected at a retail LPM, Guangdong, China, December 2015–July 2018*

Type of samples	No. samples	Median monthly positive rate, % (95% CI)		
		H5	H7	H9
Poultry samples†	1,239	0.0 (0.0–2.5)	5.8 (0.0–15.0)	64.6 (55.0–67.5)
Oropharyngeal	1,239	0.0 (0.0–2.5)	5.8 (0.0–13.3)	60.0 (52.5–67.5)
Cloacal	1,239	0.0 (0.0–2.5)	0.0 (0.0–2.5)	20.0 (13.2–26.5)
Environmental samples	1,734	6.2 (0.0–11.8)	4.2 (1.6–22.0)	73.8 (60.0–79.4)
Poultry holding zone‡	965	2.9 (0.0–6.3)	3.0 (0–20.0)	68.2 (56.0–75.9)
Fecal droppings	424	0.0 (0.0–7.7)	0.0 (0.0–11.1)	58.3 (50.0–66.7)
Drinking water	364	0.0 (0.0–8.3)	3.3 (0.0–15.4)	83.3 (66.7–100.0)
Poultry feed	177	0.0 (0.0–11.1)	0.0 (0.0–9.1)	50.0 (33.3–70.0)
Poultry slaughtering zone	457	2.2 (0.0–25.0)	6.1 (0.0–22.2)	78.6 (59.5–87.5)
Defeathering machine	250	0.0 (0.0–20.0)	0.0 (0.0–20.0)	86.2 (70.0–100.0)
Defeathering area	207	0.0 (0.0–25.0)	0.0 (0.0–12.5)	73.2 (50.0–87.5)
Poultry selling zone	194	27.9 (0.0–50.0)	0.0 (0.0–14.3)	91.2 (60.0–100.0)
Chopping board	141	33.0 (0.0–50.0)	0.0 (0.0–25.0)	100.0 (71.4–100.0)
Display table	53	0.0 (0.0–66.7)	0.0 (0.0–14.3)	92.9 (25.0–100.0)
Air§	118	0.0 (0.0–25.0)	0.0 (0.0–16.7)	75.0 (50.0–100.0)

*AIV, avian influenza virus; LPM, live poultry market; qRT-PCR, quantitative reverse transcription PCR.
†A positive poultry sample may detect AIV in the oropharyngeal samples, cloacal samples, or both by qRT-PCR.
‡Environmental swab specimens were collected within the same poultry stall at LPMs but may not be from the same cage where the chickens were sampled. Fecal droppings were collected from the ground or cages, drinking water was collected from the water troughs, and poultry feed was sampled from the surface of the bowls or feeders.
§A positive air sample may be positive for AIV by qRT-PCR in any of the 3 size fractions collected by a NIOSH sampler (*11*). Two to 6 NIOSH samplers were applied monthly to sample air at the retail markets.

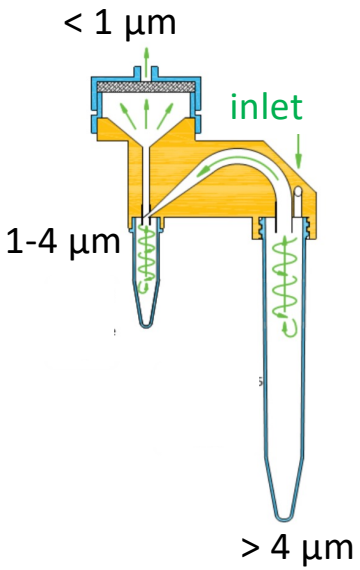
Correlation between AIV detection rates in poultry and environmental samples at live poultry markets



Isolation of H5N6, H7N9 and H9N2 avian influenza A viruses from air sampled at live poultry markets in China, 2014 and 2015

J Zhou[1,2], J Wu[2,3], X Zeng[2,3], G Huang[3], L Zou[3], Y Song[3], D Gopinath[1], X Zhang[3], M Kang[3], J Lin[3], BJ Cowling[4], WG Lindsley[4], C Ke[3], JSM Peiris[1], H Yen[1]

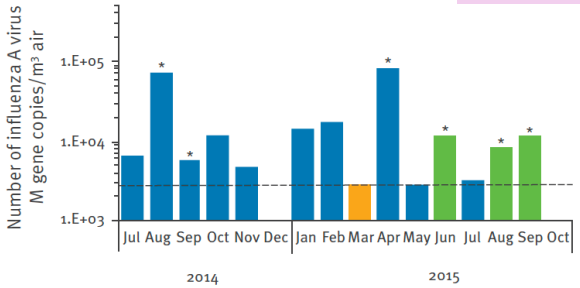
NIOSH bioaerosol sampler

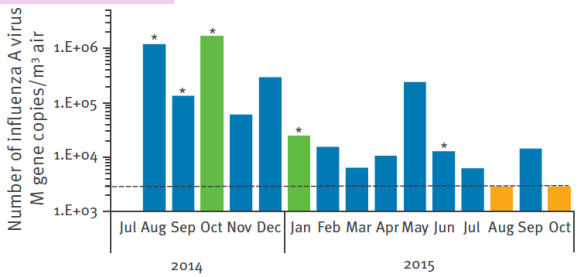




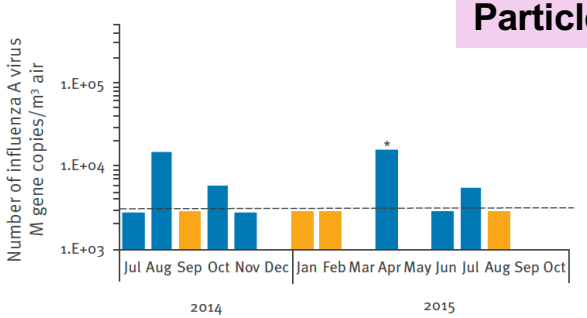


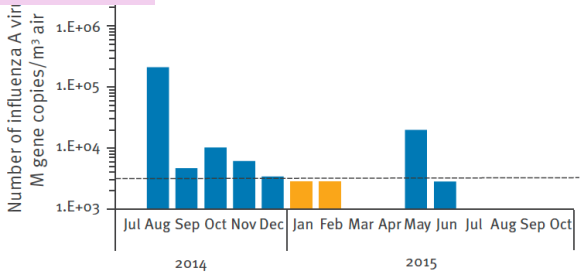
Particles > 4 µm



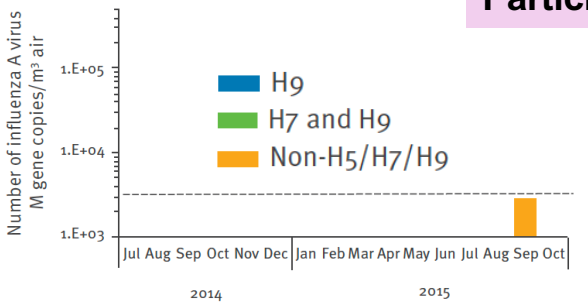


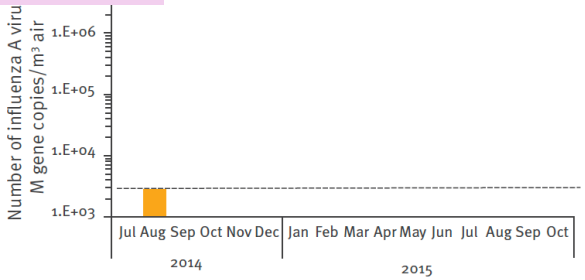
Particles 1-4 µm





Particles < 1 µm

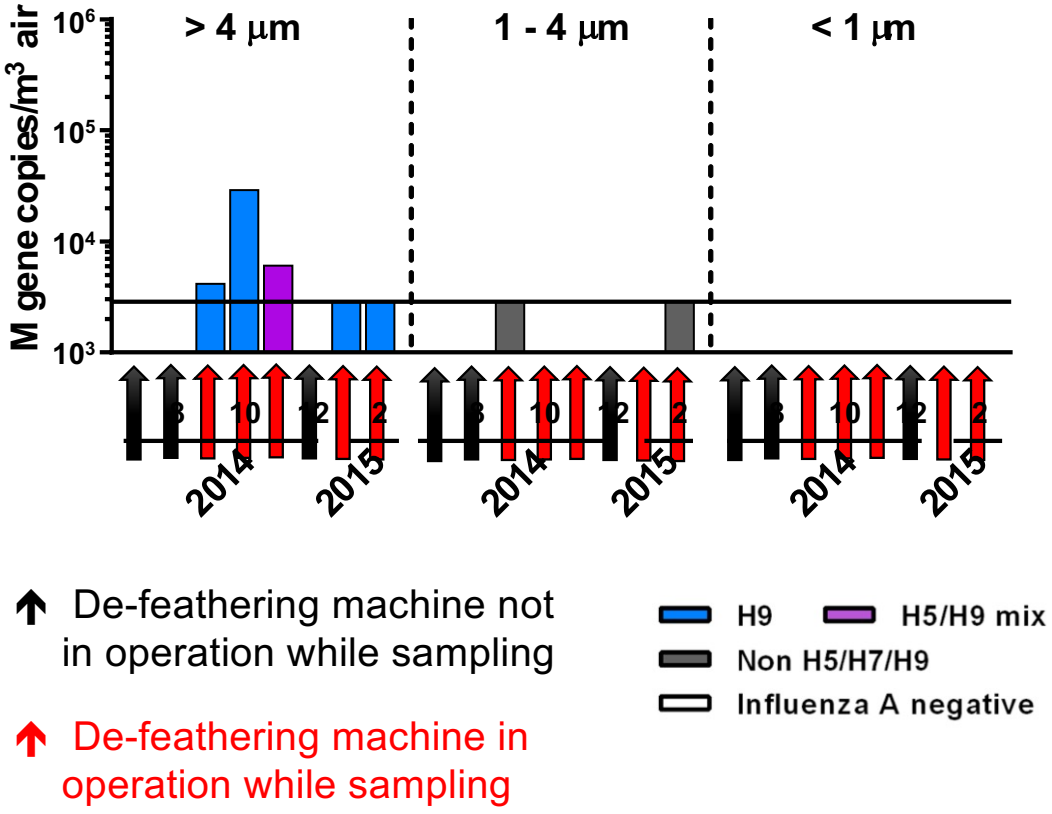




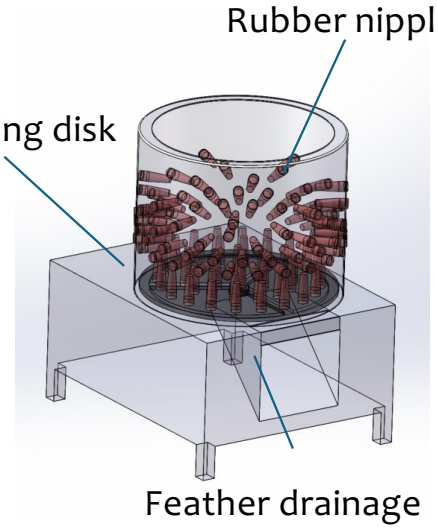
PMID: 27608369

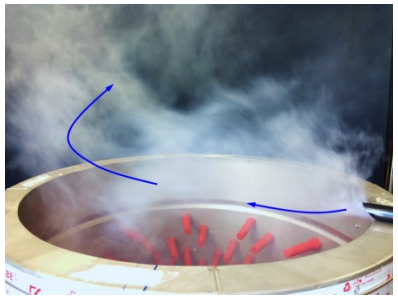
The use of de-feathering machines may facilitate generation of infectious virus-laden particles in air



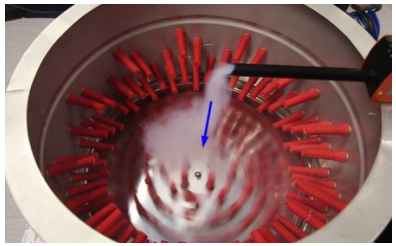


Airflow characteristics of the de-feathering machine during operation

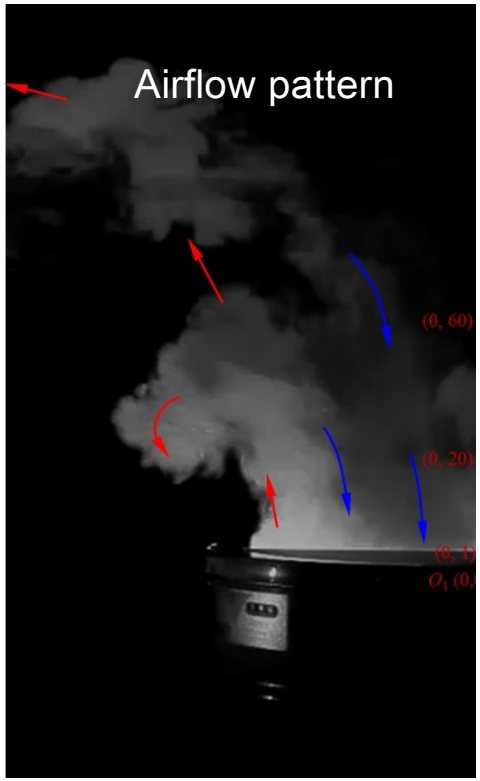


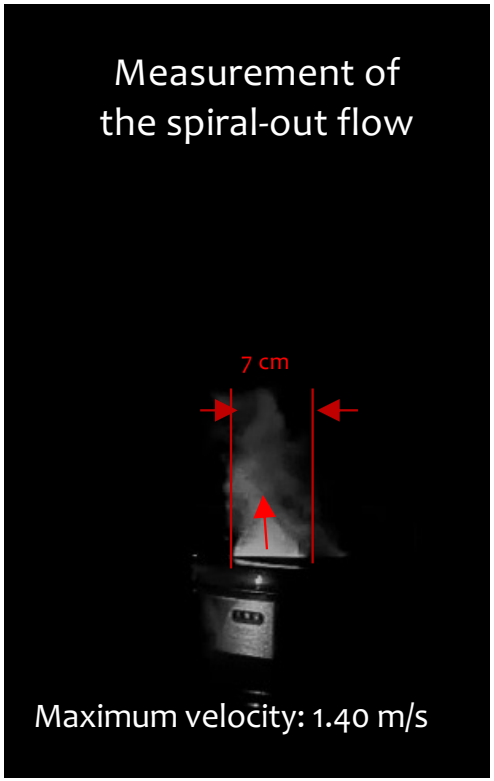


Flow spirals out of the plucker



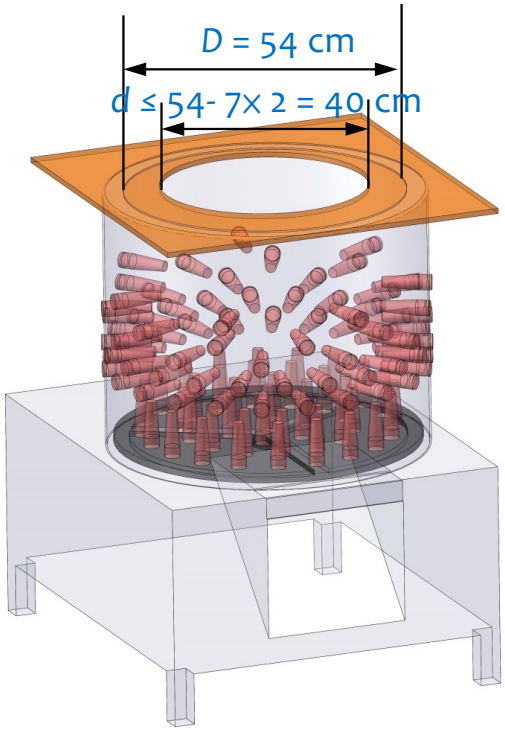
Downward flow towards the disk center





PMID: 31067261

Design of a partial lid for the de-feathering machine to reduce aerosol release



A lid is designed to block the upwards air current with an opening for workers' operation.



The partial lid suppresses the flow: it does not efficiently travel as high as when there is no lid..

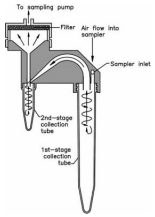
Downwind spread of avian influenza virus via airborne particles from an urban wholesale poultry market

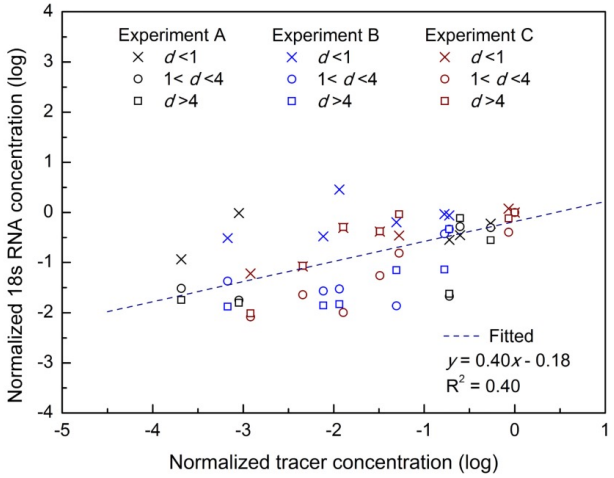
CFD modeling indicated the combined effect of wind direction and surrounding buildings on the downwind spread of influenza virus



Validation

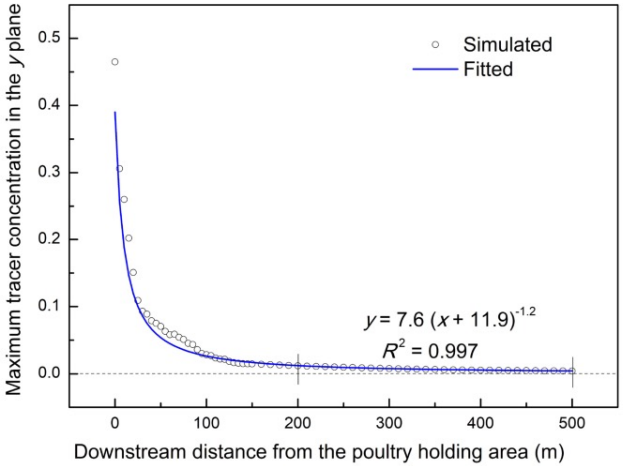
Correlation between measured 18S RNA concentration and predicted tracer concentration





Prediction

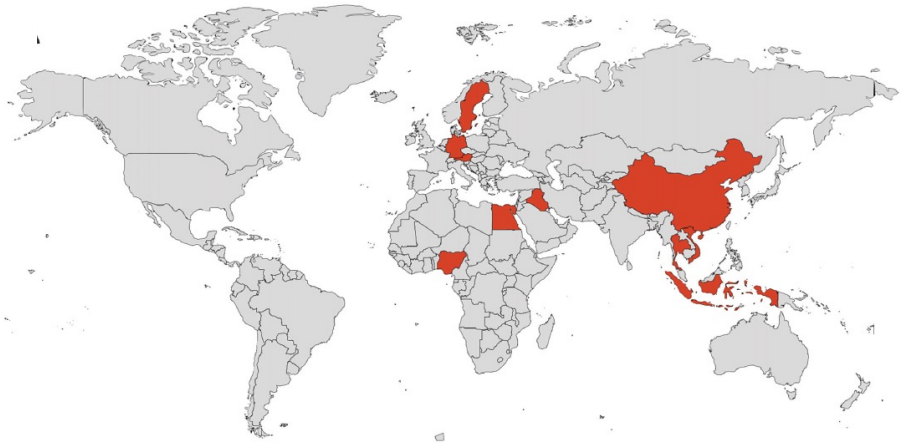
Modelled tracer concentration decay in the downstream direction

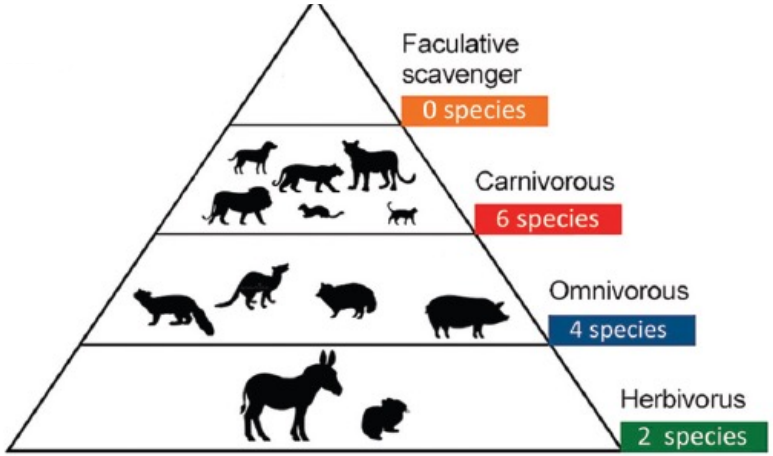


The concentration of viral RNA detected at the poultry holding area was 4.4×10^5 copies/m^3 and was predicted **to drop below 100 copies/m^3 after a downwind distance exceeding 5.9 km.**

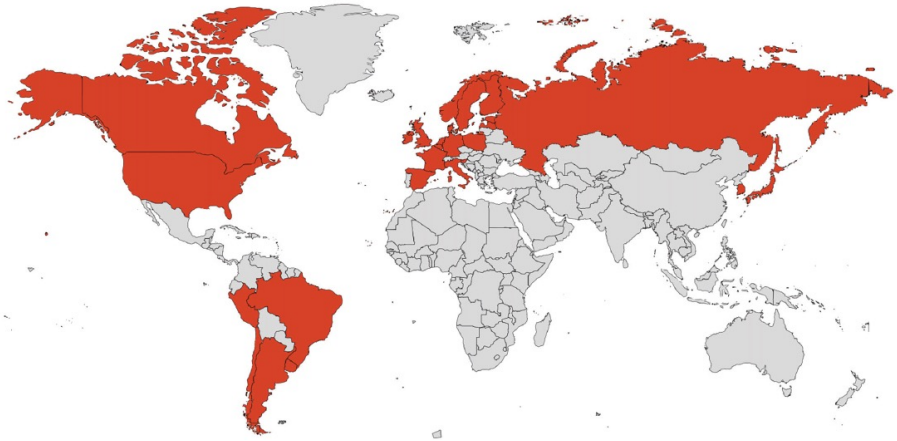
The H5N1 panzootic

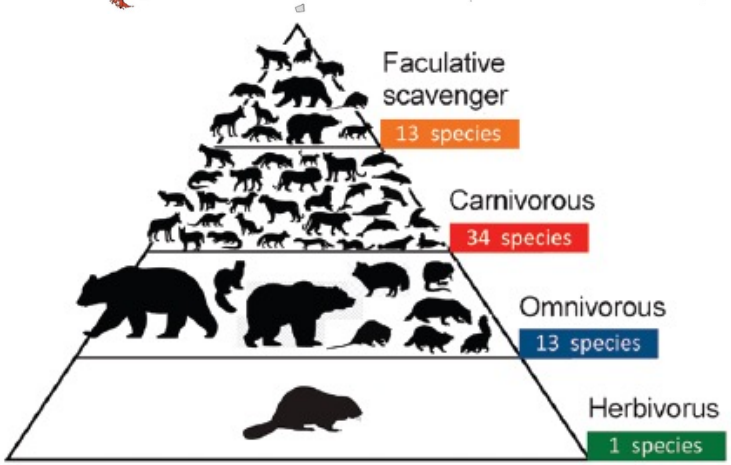
2003-2019





2020-2023

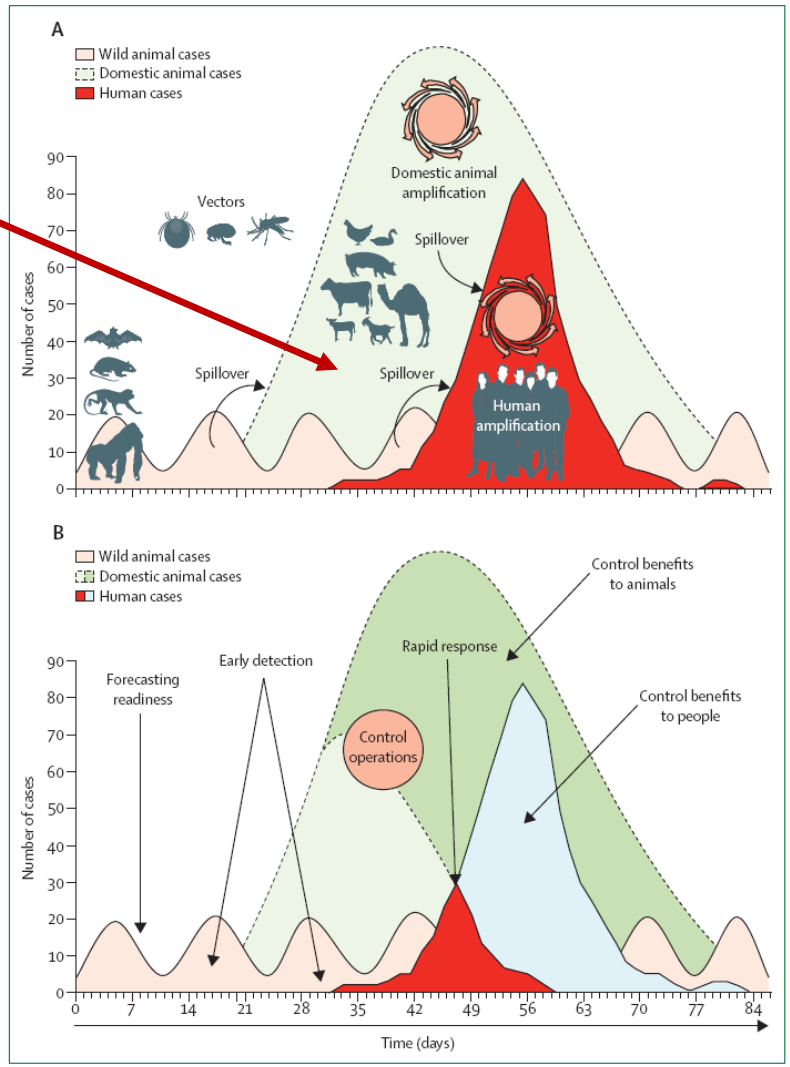




PMID: 38407173

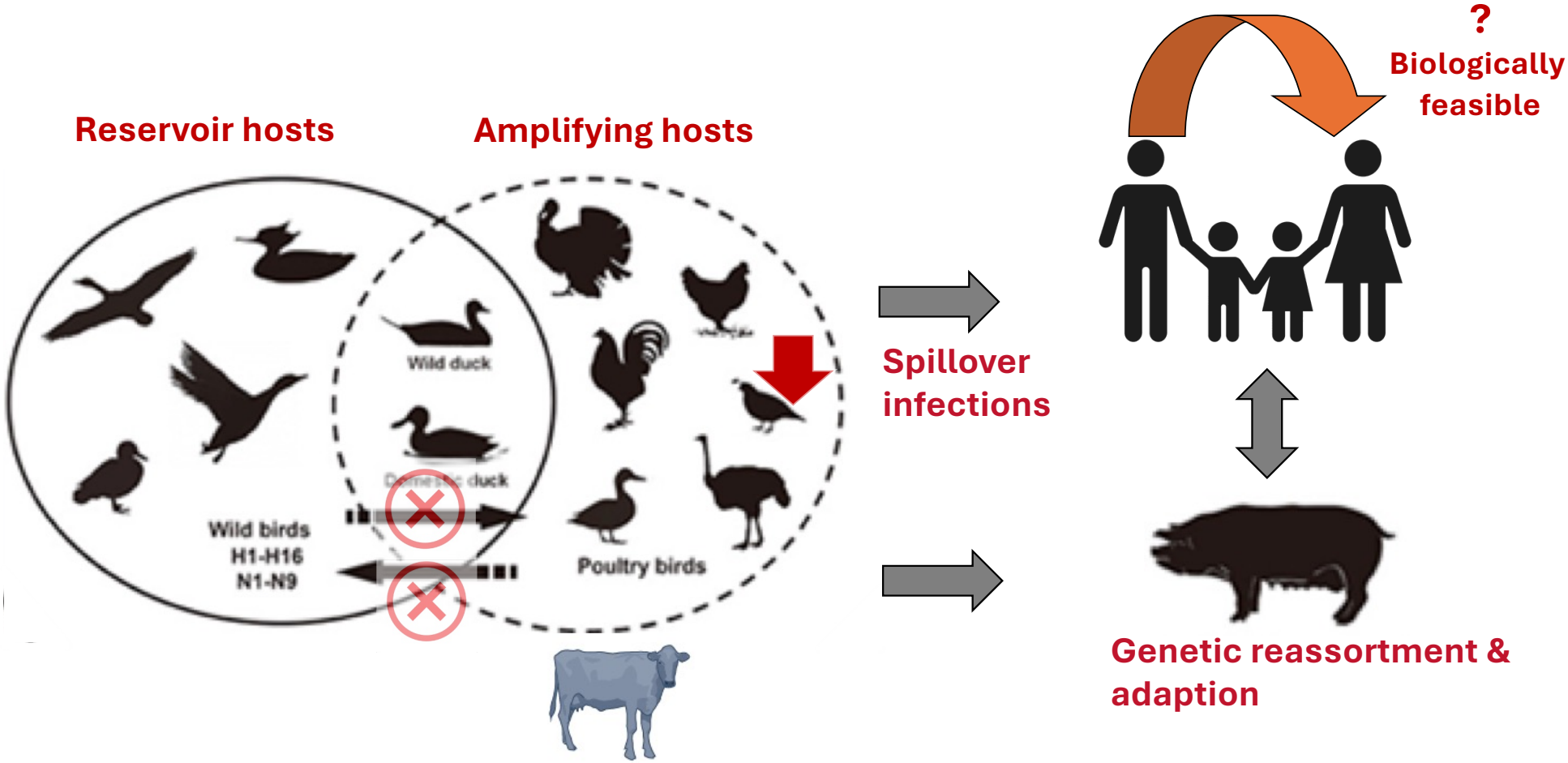
Effect of early detection and control efforts

We have missed some precious opportunities already!



Karesh et al., Lancet 2012

Actions needed to prevent transmission between the reservoir and amplifying hosts



Questions?

hyen@hku.hk