Threats posed by H7N9 virus, drivers of emergence and options for risk-reduction

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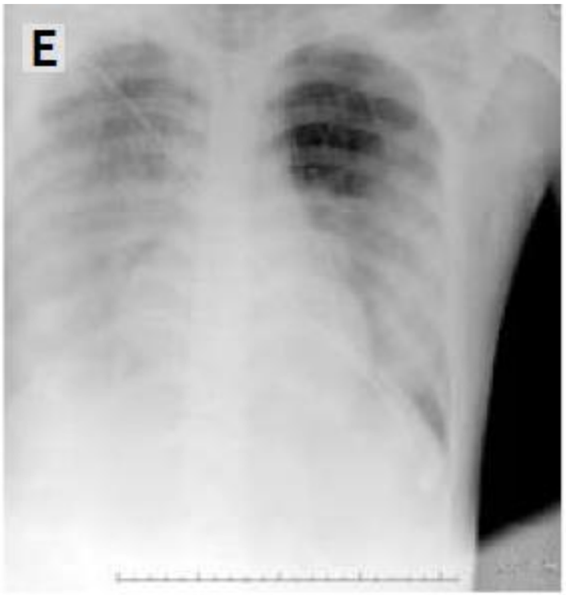
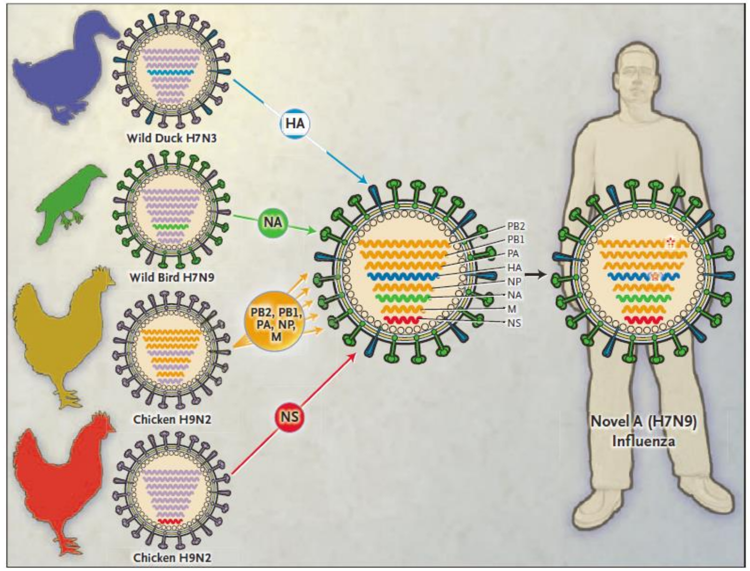
Emergence and origins of the avian flu H7N9 virus





February 2013 Investigation initiated by three adult members of the same family with severe pneumonia

Biologically important amino acid mutations

PB2 E627K: *Mammalian adaptation, seen only with some human isolates*

HA: S138A; T160A; G186V, Q226L *Human receptor binding*

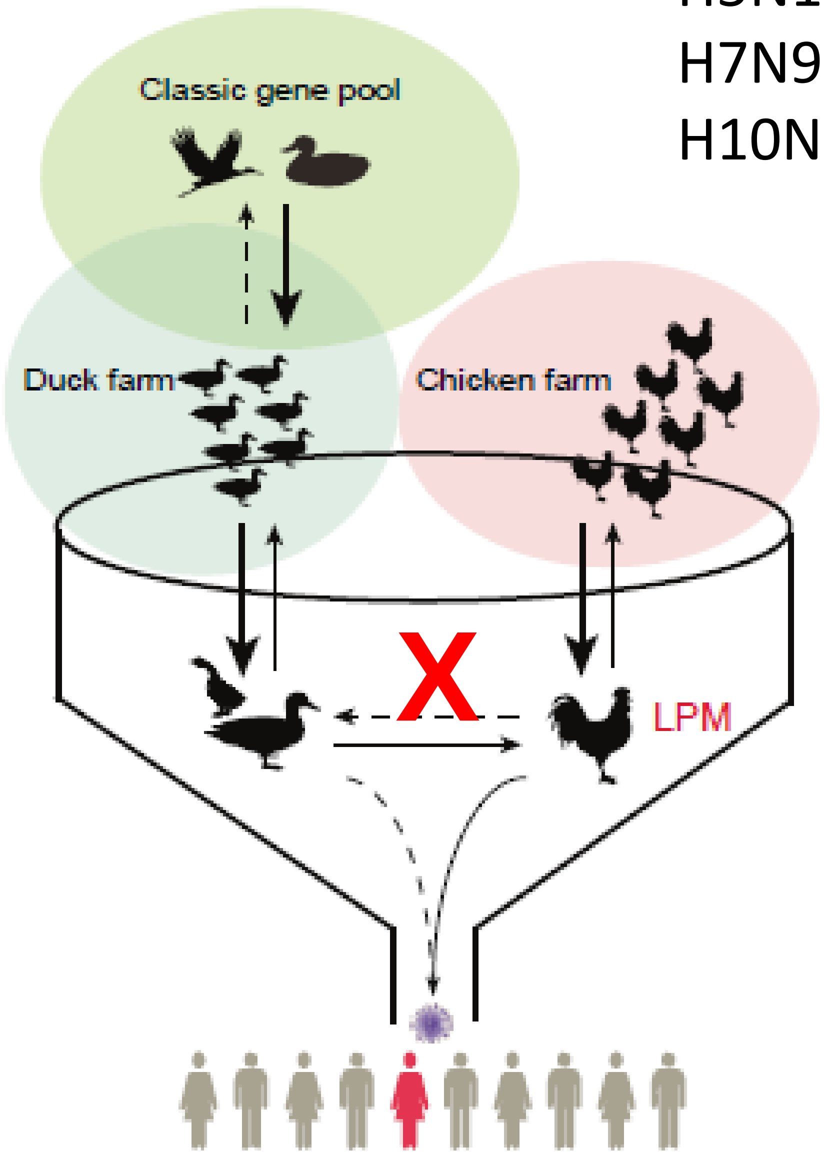
NA: *stalk deletion* R292K *NI resistance*

M2: S31N *amantadine resistance*

Gao et al NEJM 2013; Morens et al NEJM 2013; Kagayama et al Eurosurv 2013

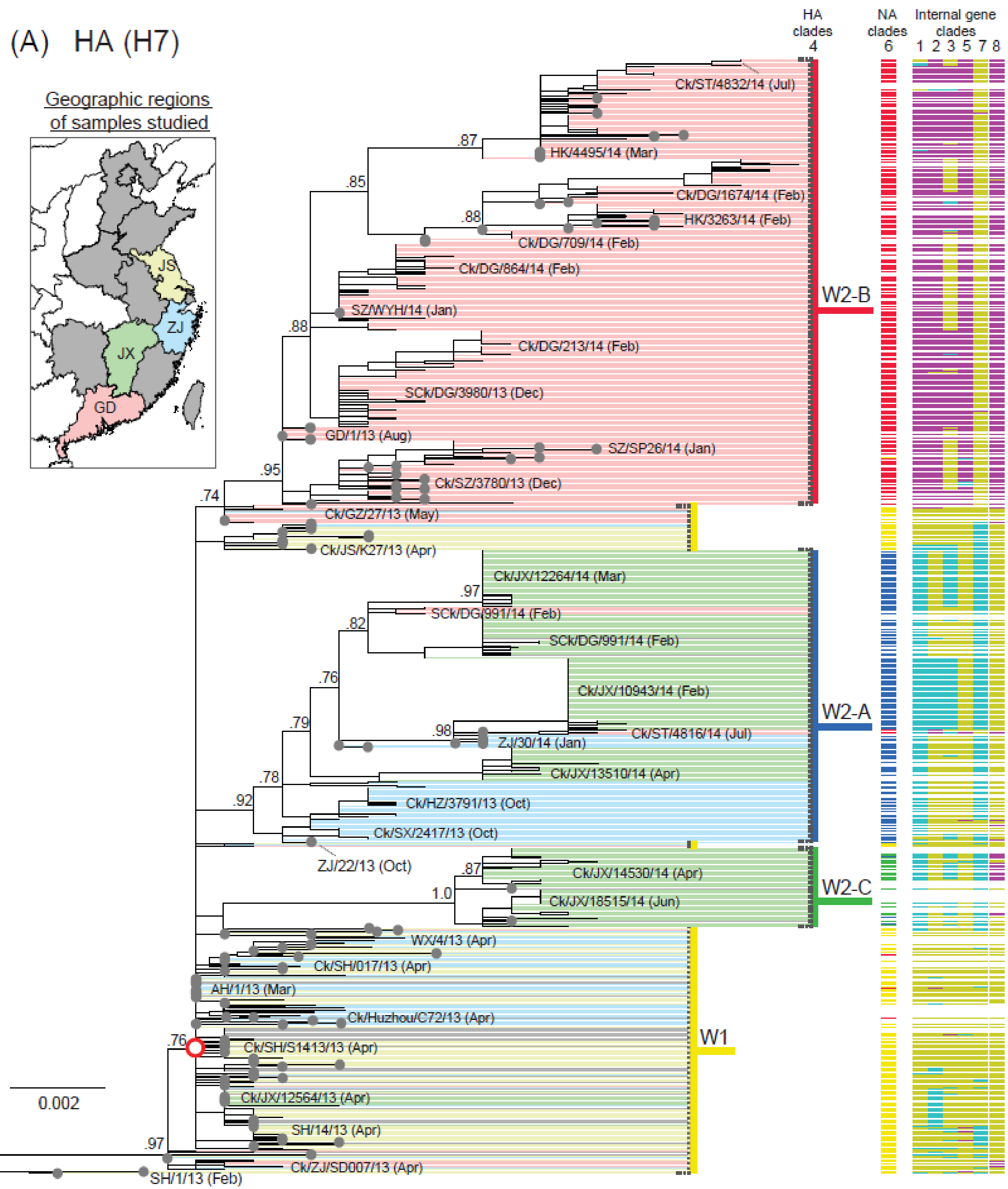
Emergence of new influenza viruses: Prevention at source

H5N1: Guan et al PNAS 1999
H7N9: Lam et al Nature 2013
H10N8: Qi et al 2014; Ma et al 2014



Separate aquatic poultry from terrestrial poultry in wholesale and retail marketing systems

Ma et al MBio 2014



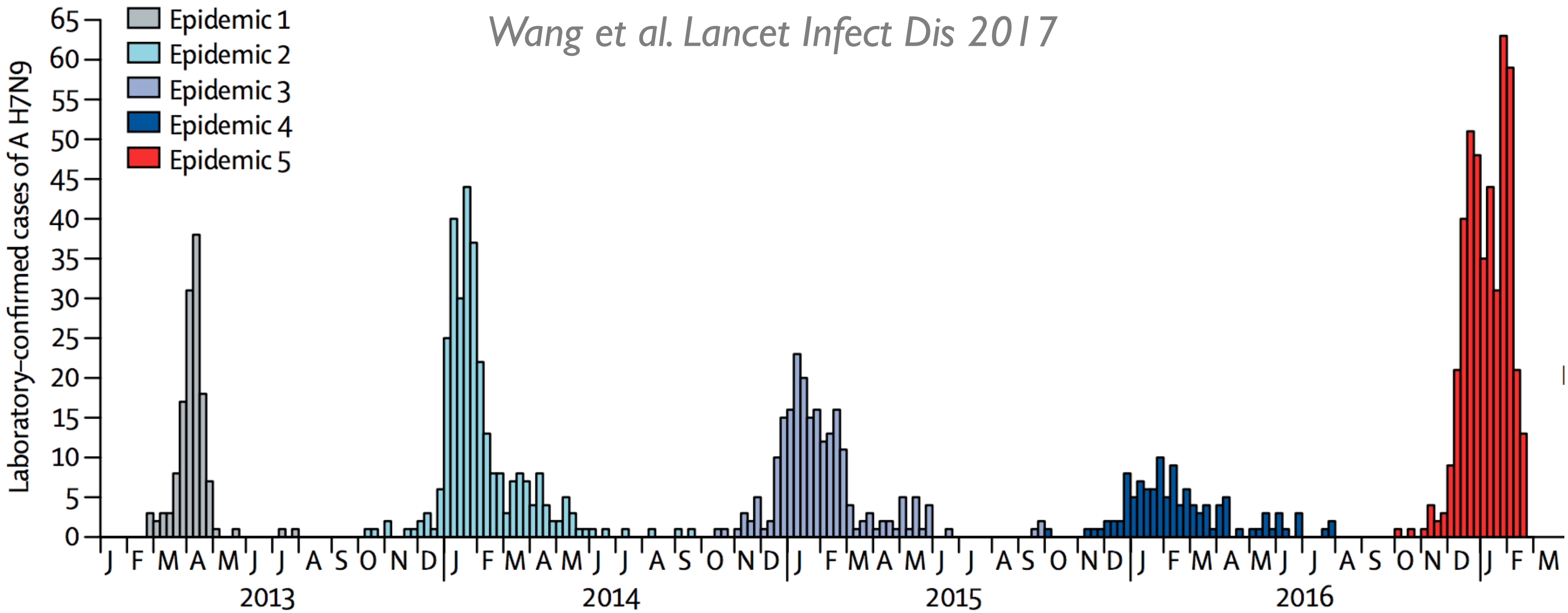
Wave1 No clear selection of evolutionary direction.

Wave2

- Approx. 3% positive in chicken in LPM
- Derived from wave 1
- Three geographically distinct clades: ladder-like topology → caused by localized transmission → driven by poultry movement.
- New reassortments with H9N2 internal genes
- Human viruses reflect viruses in poultry.
- Avian virus molecular signatures in PB2 remain avian, NA remains NAI sensitive;

Lam et al Nature 2015

H7N9 human cases across five waves



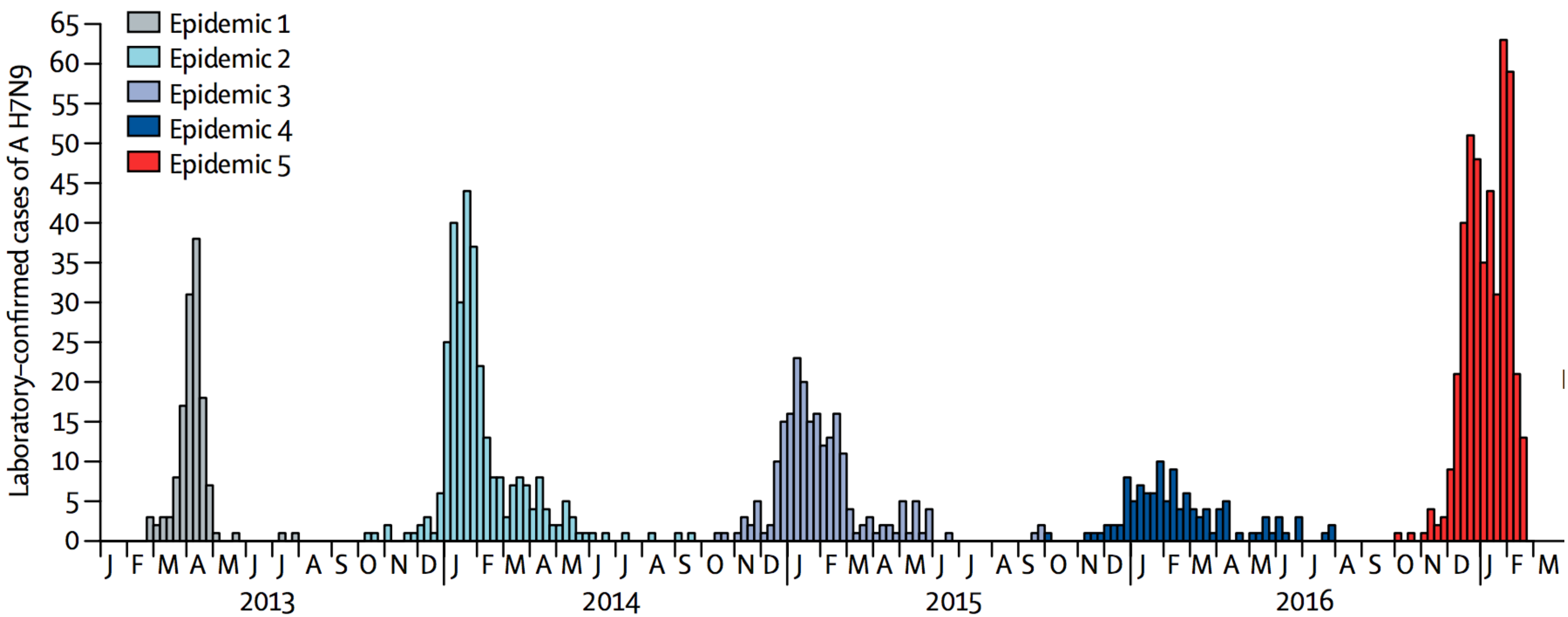
Provinces/ municipalities	13	16	14	15	28

Changes in recent waves:

- Increase in rural cases
- No change in severity or fatality rates

Zhou Lei ISIRV AVG June 2017

H7N9 human cases across five waves



Mild cases detected through sentinel ILI surveillance (n= 82/1220)

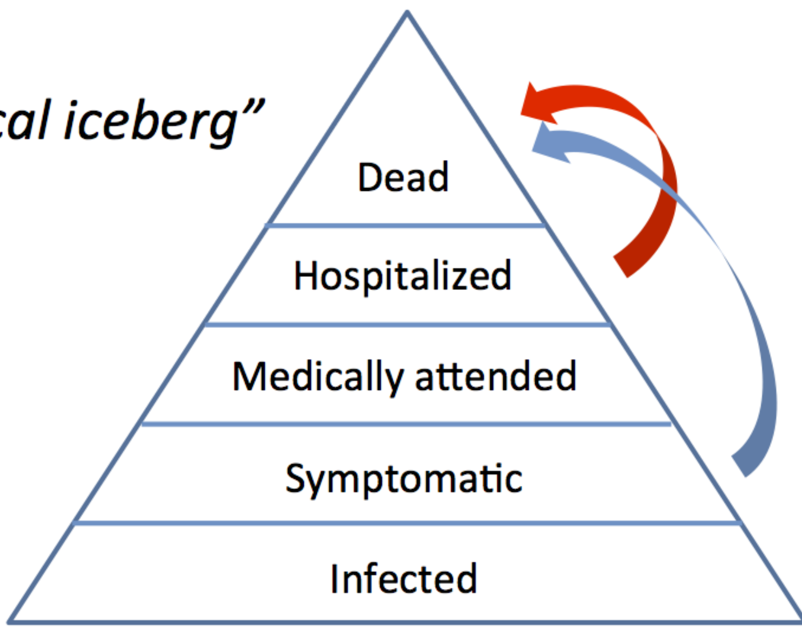
Wave 1	Wave 2	Wave 3	Wave 4	Wave 5
10 (7%)	33 (11%)	27 (12%)	7 (6%)	5 (3%)

Wang et al. Lancet Infect Dis 2017

Severity of H7N9 human infections

Influenza virus	Fatality risk	
	Hospitalized cases	Symptomatic cases
A(H1N1)pdm09	5%-20%	~0.01%
A(H7N9)	36%-48%	0.07%-0.5%
A(H5N1)	65%	-
A(H5N6)	75%	-

"Clinical iceberg"



The hospitalization fatality risk represents the risk of death for hospitalized cases (36%-48%)

The symptomatic case fatality risk represents the risk of death for symptomatic cases

Based on medically attended cases adjusted for health seeking behaviour

Wong et al. 2013 Epidemiology; Yu et al. 2014 Lancet; Cowling et al. 2013 Lancet; Feng et al. 2014 Eurosurveil; Wong et al. 2015 Am J Epidemiol; Qin et al. 2015 Clin Infect Dis; Jiang et al. 2017 Clin Infec Dis

Good News – Bad News

- Case fatality ratio much lower than feared

- Number of zoonotic infections is much greater than supposed→ increased risk of virus adaptation to human transmissibility

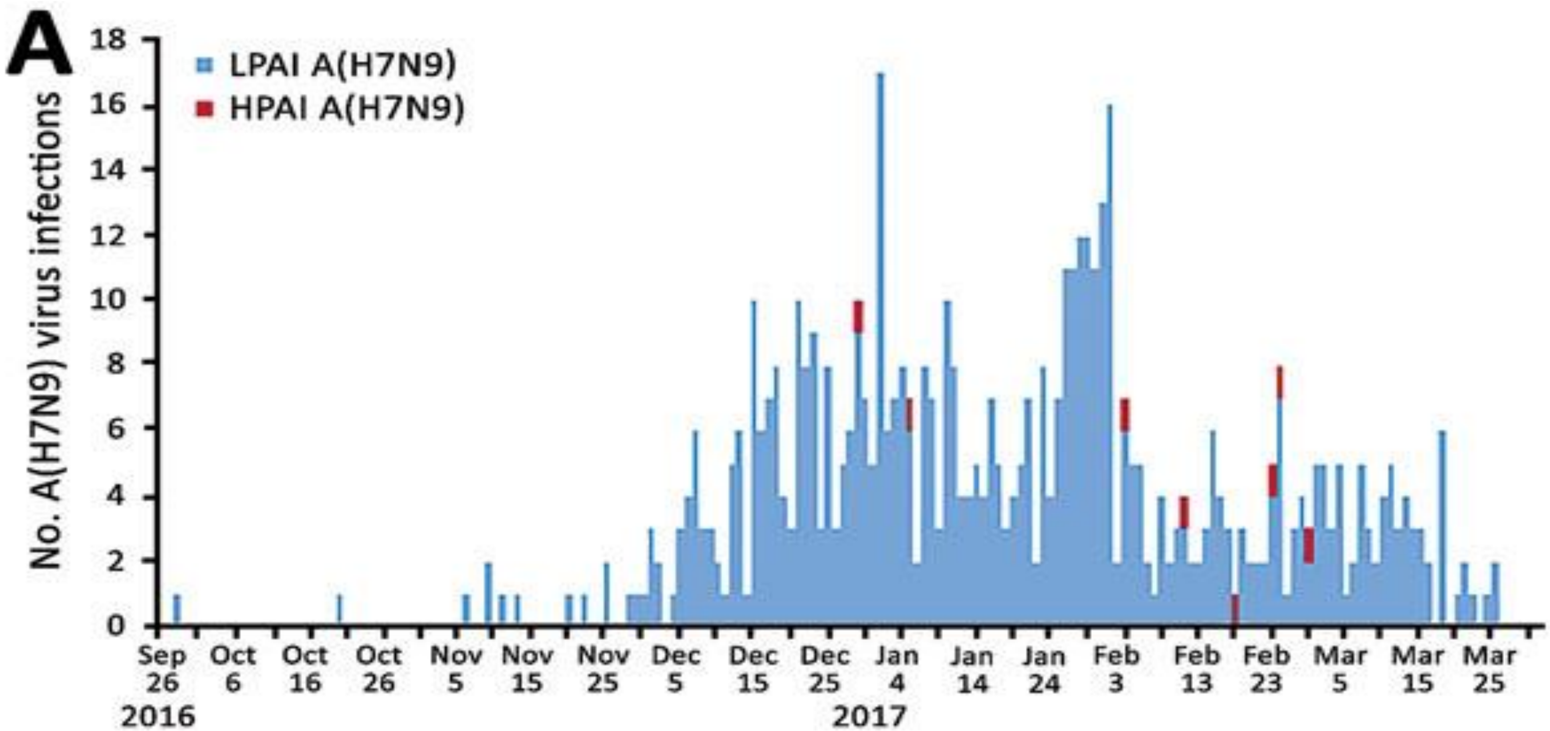
Clusters of avian influenza in China: H7N9 vs. H5N1

	Sporadic cases & index cases	Secondary cases	P value
H7N9	**N=407**	**N=19**	
Age (median, range)	59 (0, 91)	31 (3, 97)	<0.001
Risk of ICU admission	70.6%	33.3%	0.007
Fatality risk	41.2%	27.8%	0.33
Relative risk of infection in blood related contacts (95% CI)		0.8 (0.33, 1.97)	
H5N1	**N=626**	**N=89**	
Age (median, range)	18 (0,86)	16 (0, 51)	0.13
Fatality risk	61.6%	54.2%	0.285
Relative risk of infection in blood related contacts (95% CI)		8.96 (1.3-61.9)	

Qin et al CID 2015

Highly pathogenic Avian Influenza H7N9

- First detected in poultry in LPM in in Guangdong in November 2016
- First human cases detected with illness onset on Dec 17th 2016. and Jan 5 2017, in Guangdong Province. Additional patients in February and later in Hunan and Guangxi



Zhou et al EID 23: (8): online

Emergence of HPAI H7N9

Analysis of LPAI and HPAI H7N9 viruses from Guangdong Province 2016/17 suggests that

- the HPAI viruses are monophyletic and emerged from the Yangtze River Delta lineage
- Molecular clock analysis suggests HPAI emergence was around March 2016 (range Dec 2015 – July 2016).
- NA gene has diverse origins from both Yangtse River and Pearl River Delta lineages → after the HPAI emergence in the Yangtse lineage virus, there was co-circulation and reassortment with LPAI viruses from the Pearl River Delta viruses.

Su W, H Yen –collaboration with Guangdong CDC X Mao, Z Zhang, Y Song, C Ke.
J Infect – on line 2017.

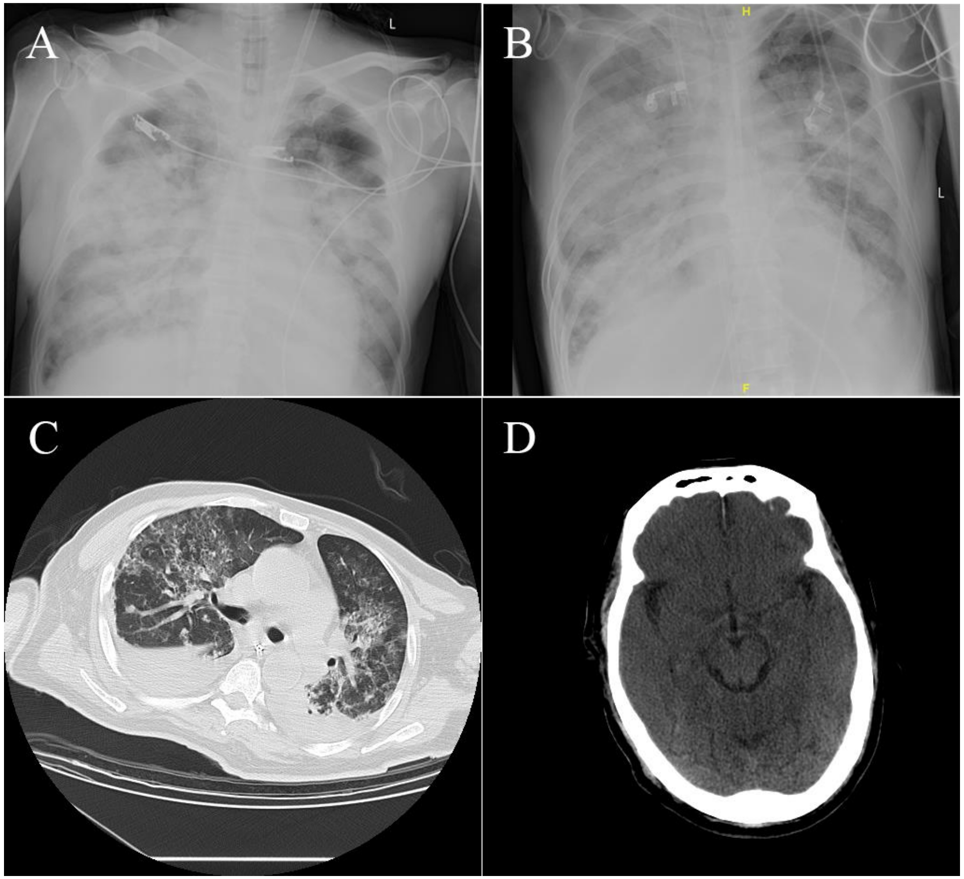
HPAI H7N9: Patient No 1

1st Affiliated Hospital of Guangzhou Medical University

56 yr old male w diabetes & hypertension

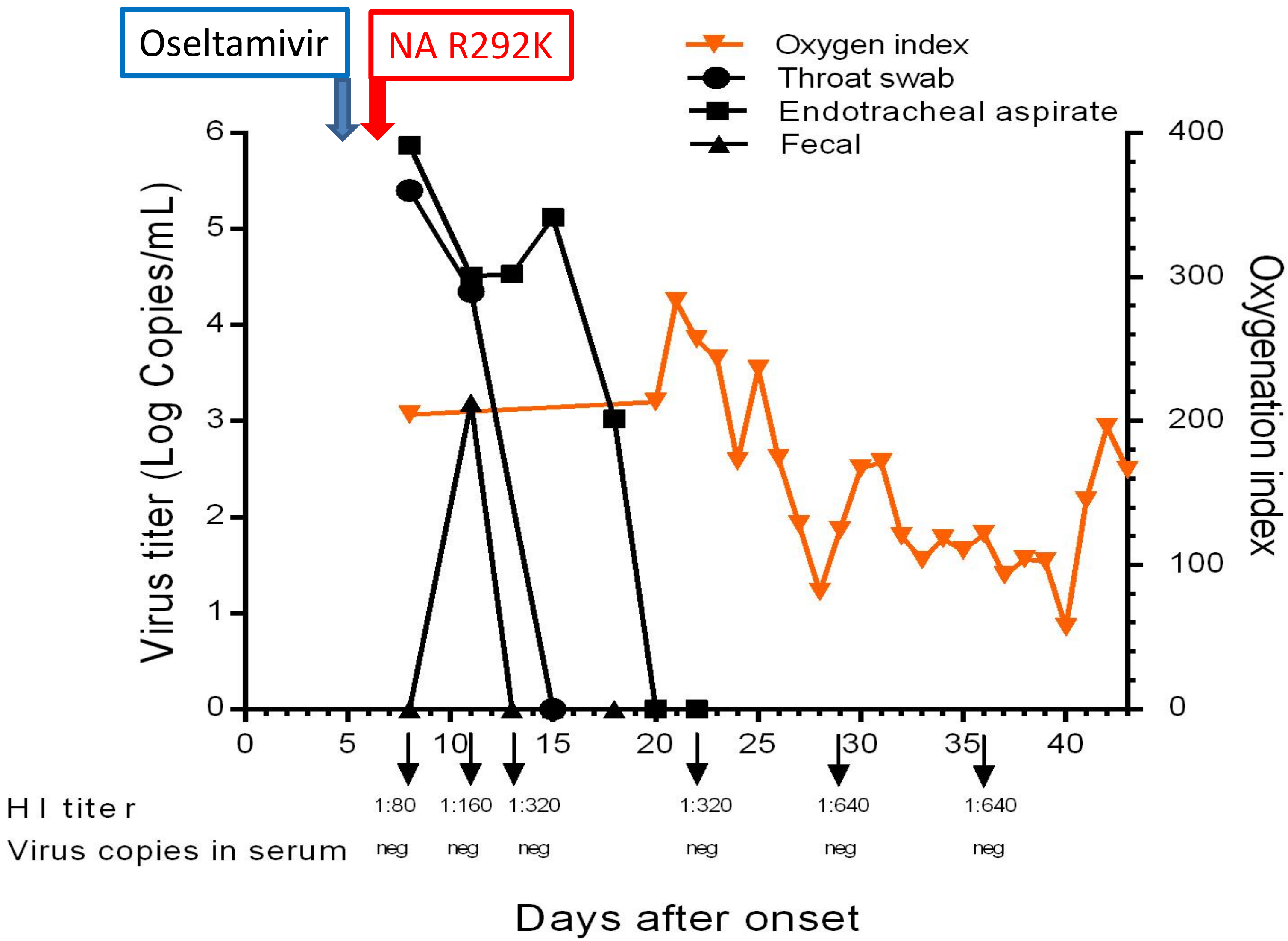
Exposure to sick backyard poultry

HA cleavage site
PEVPKRKRTAAR/G



Ke et al EID 2017; 23; - online

Viral load kinetics



Ke et al EID 2017; 23; - online

- Of 28 human HPAI H7N9 isolates,
  - - 7 (25%) have mutations that reduce sensitivity to neuraminidase inhibitors; of these 5 have NA 292K mutations (data from China CDC)
- R292K mutations associated with adverse clinical outcome (Hu et al Lancet 2013)
- Virus isolate will under-estimate true incidence R292K mutation mBio 2013(Yen et al)

Comparison of patients with HPAI vs LPAI H7N9

(Guangdong Province: Nov 1st 2016 – March 31st 2017)

	HPAI (n=9) (%)	LPAI (n=51) (%) Guangdong	P value
Symptoms			
Fever	8/9 (89%)	47/51 (92%)	0.57
Cough	8/9 (89%)	44/51 (86%)	1.0
Sore throat	1/9 (11%)	13/51 (37%)	0.67
Muscle pain	4/9 (44%)	10/51 (20%)	0.19
Diarrhoea	0/9 (0%)	2/51 (4%)	1.0
Raising backyard poultry#	7/9 (78%)	15/51 (29%)	0.009
Exposure to sick/dead poultry	6/9 (67%)	5/50 (10%)	0.001
Touched sick/dead poultry#	5/9 (56%)	5/50 (10%)	0.005
Visited live poultry markets	5/9 (55%)	31/50 (62%)	0.73

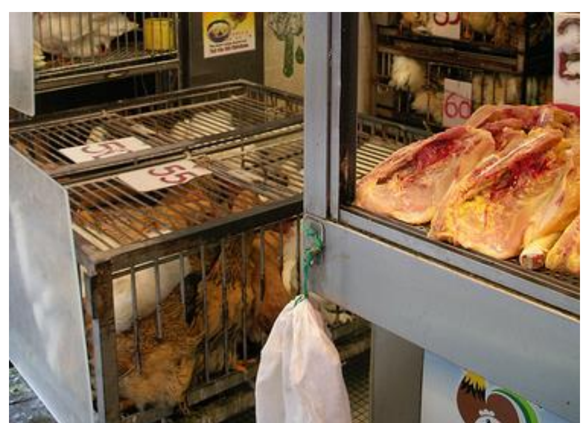
Kang et al Euro-surveillance 2017 – on line

Risk factors for zoonotic avian influenza A (H7N9) infections at the human-avian interface

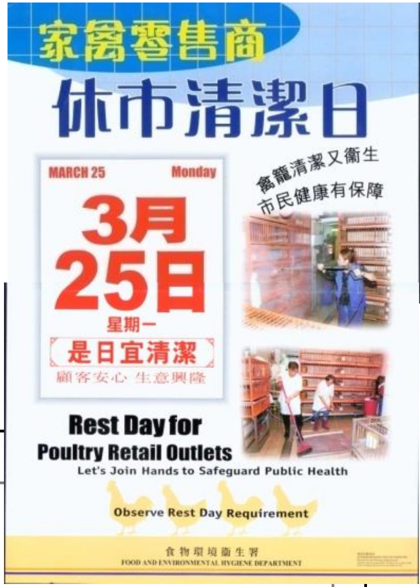
Case-control study among H7N9 patients (n=89) with age, sex, and neighborhood-matched controls (n=339):

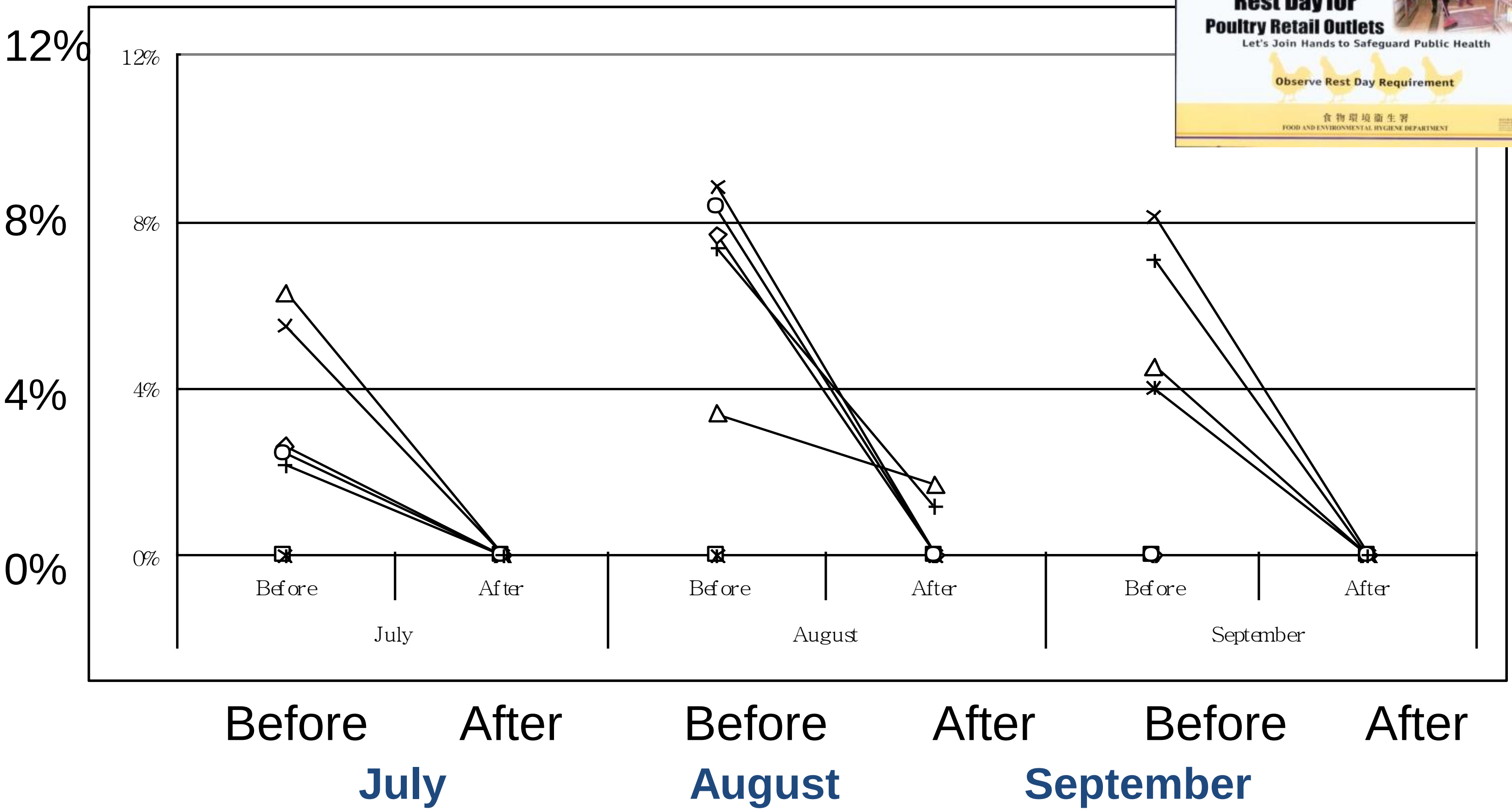
- Visited LBM (past 10 day): 67% patients versus 35% controls (mOR=5.4; 95% CI, 3.0–9.7)
- Direct or indirect contact with poultry in LBM: 33% patients versus 8% controls poultry (mOR=10.4, 95% CI, 4.9-22.0).
- Visited LMB but no direct contact with poultry: 33% patients versus 26% controls (mOR=3.0; 95% CI, 1.6-5.7).

Liu B. et al. CID 2014



Impact of “rest day”in retail markets on H9N2 isolation rates in chicken



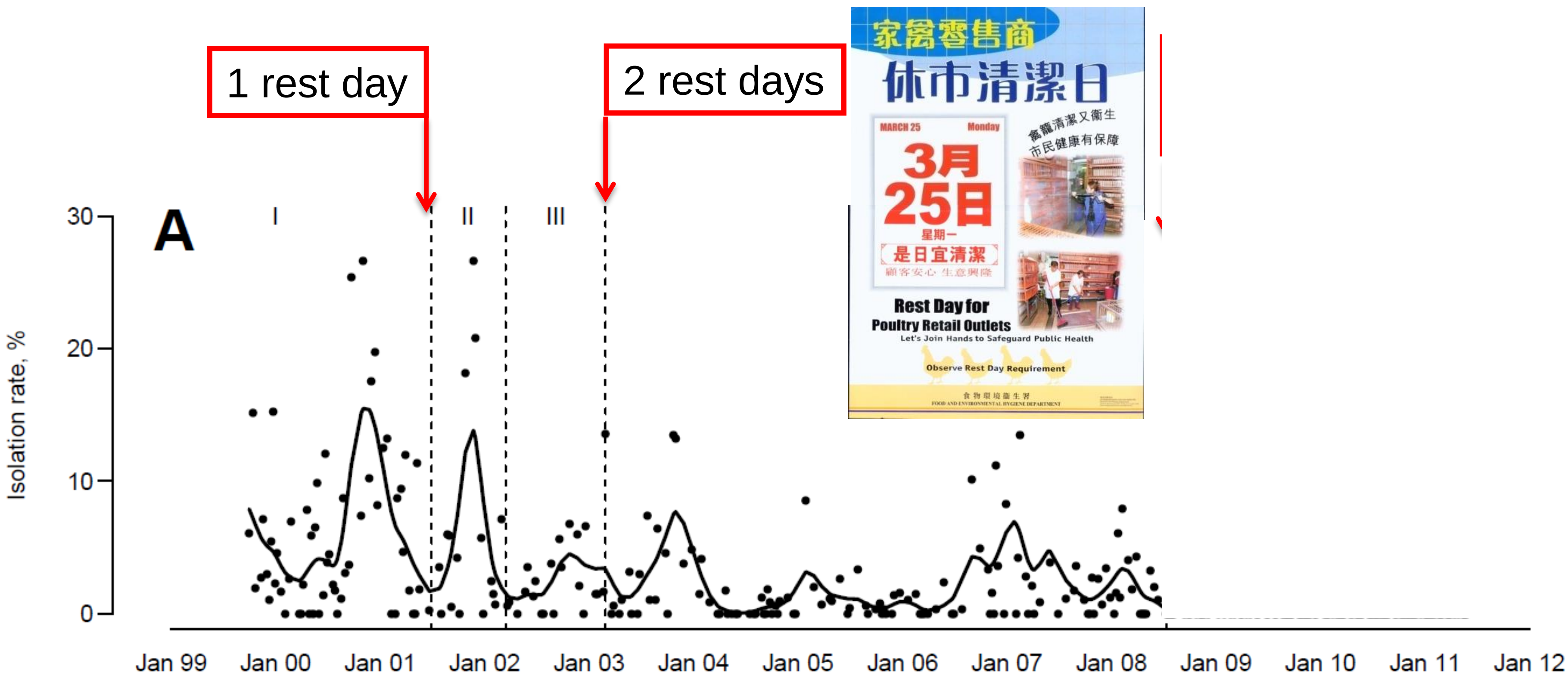


Kung et al Avian Dis 2003

Evidence based interventions in live poultry markets

Isolation rates of H9N2 viruses in chicken

1999-2011; monthly surveillance; 5-8 FEHD poutlry markets; 53,541 samples



Leung et al EID 2012

Modelling predicts effect
Pepin et al BMC Infectious Diseases 2013; 13: 592

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Liu B. et al. CID 2014

Possibility of airborne transmission?

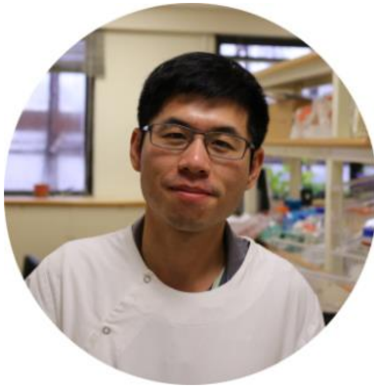
SURVEILLANCE AND OUTBREAK REPORT

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

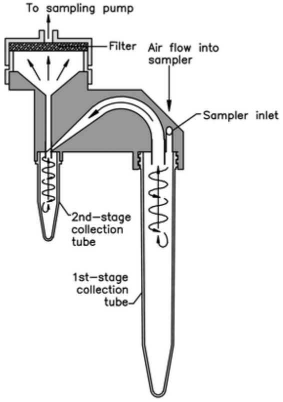
J Zhou[1,2], J Wu[2,3], X Zeng[2,3], G Huang[3], L Zou[3], Y Song[3], D Gopinath[1], X Zhang[3], M Kang[3], J Lin[3], BJ Cowling[1], WG Lindsley[4], C Ke[3], JSM Peiris[1], H Yen[1]

Eurosurveillance 2016

Yen Hui-Ling



Jie Zhou



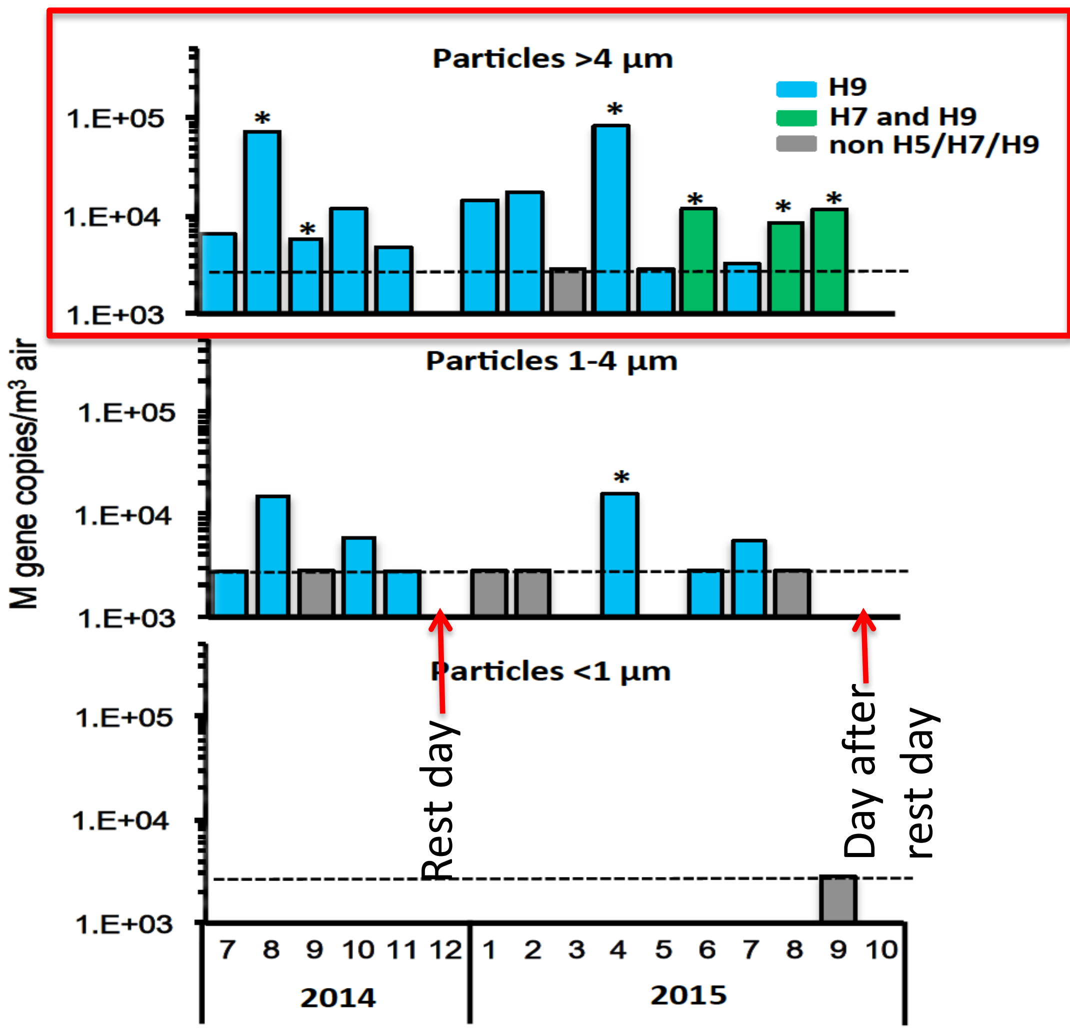
NIOSH cyclone air sampler

Coriolis cyclone air sampler

Influenza A virus M gene copy number from airborne particles of different sizes. Poultry markets, Guangzhou, 2014-15

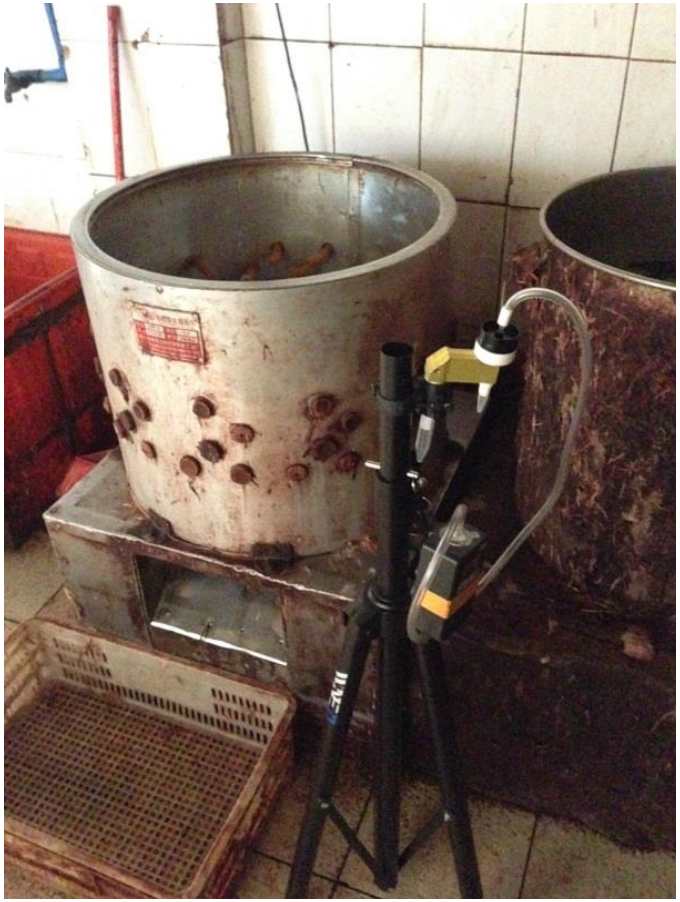
Influenza virus detection (RT-PCR, culture) by air sampling: Wholesale live poultry market

* +ve virus isolation

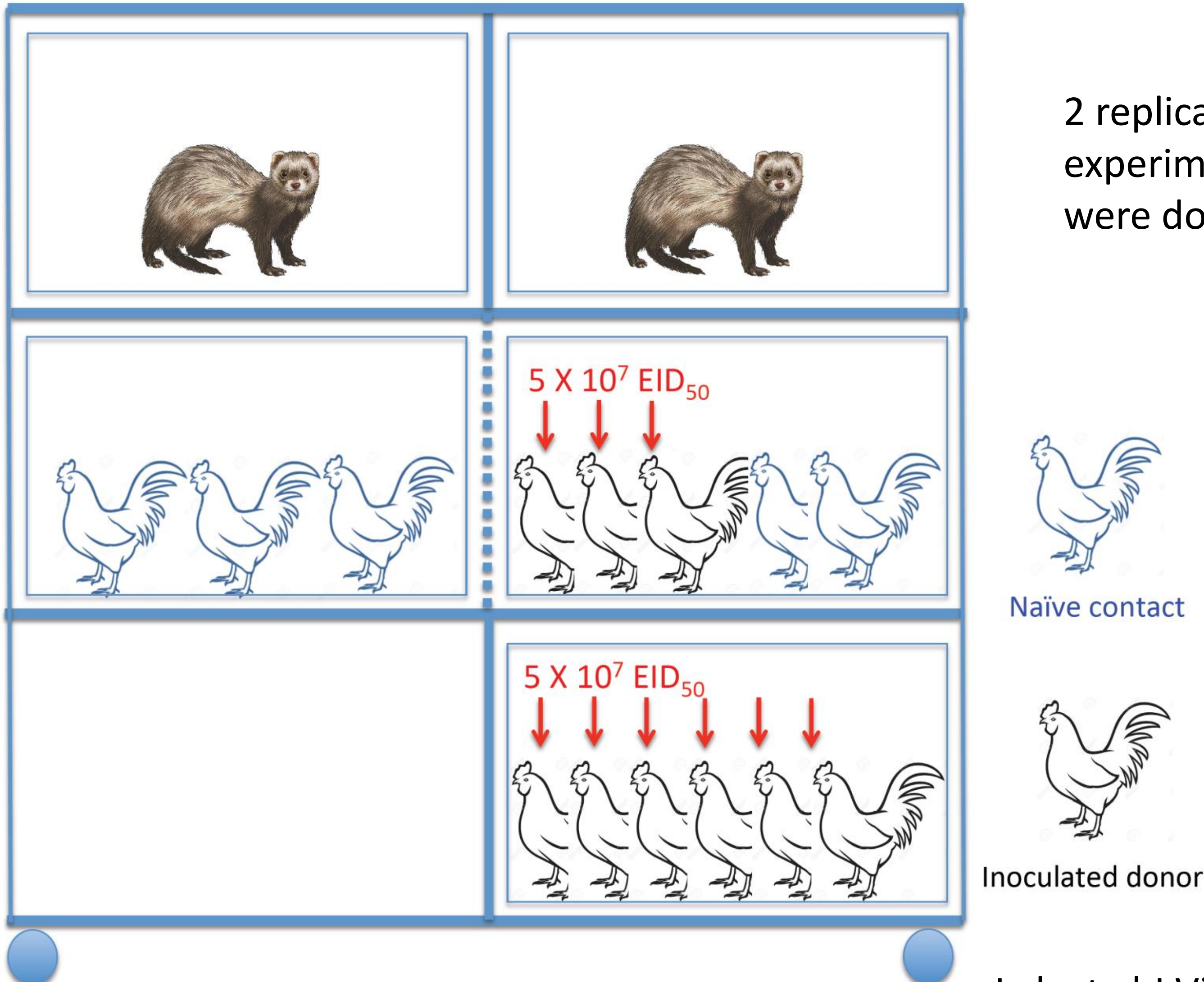


- H9N2 and H7N9 viruses detected and isolated from air sampling
- More virus in large droplets
- Correlated with environmental swab testing
- Not detected on or immediately after rest days

Virus load in air much higher in poultry market when de-feathering machine was in operation



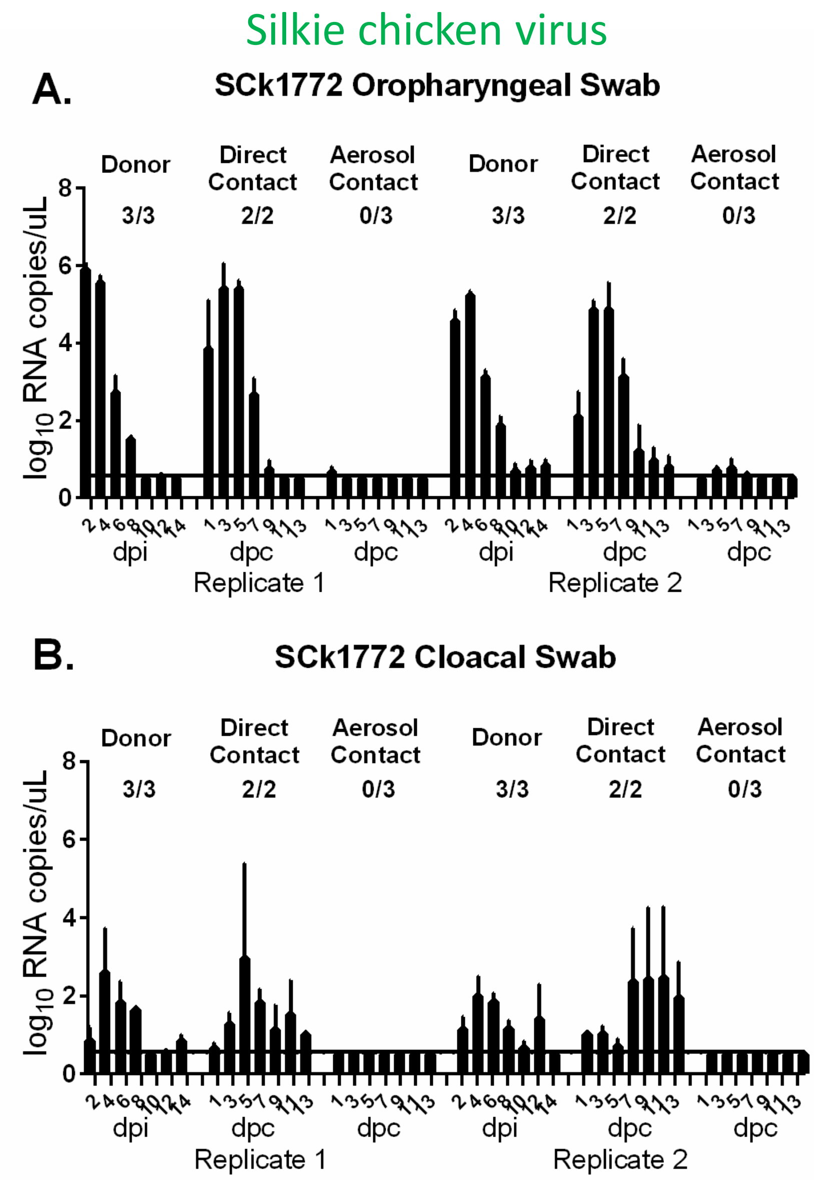
Route of transmission of H7N9 viruses in experimental settings



2 replicate experiments were done

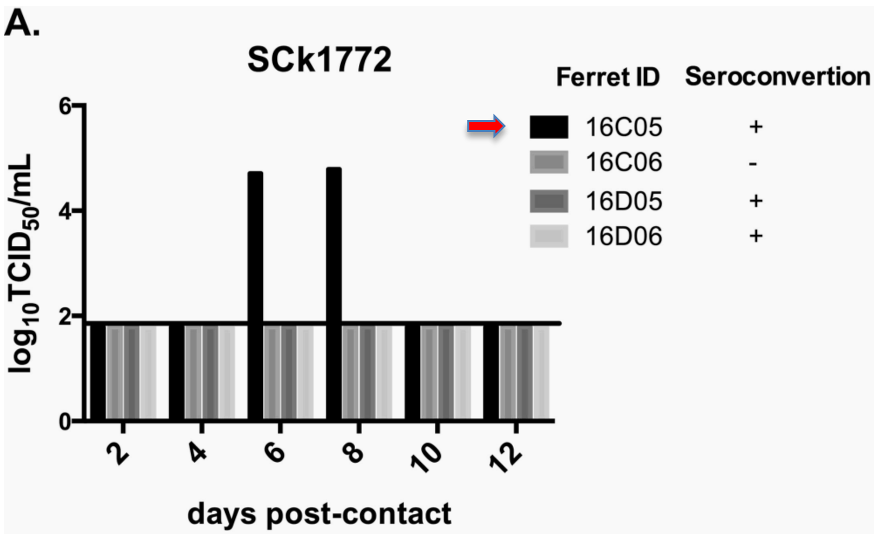
Luk et al J Virol 2015

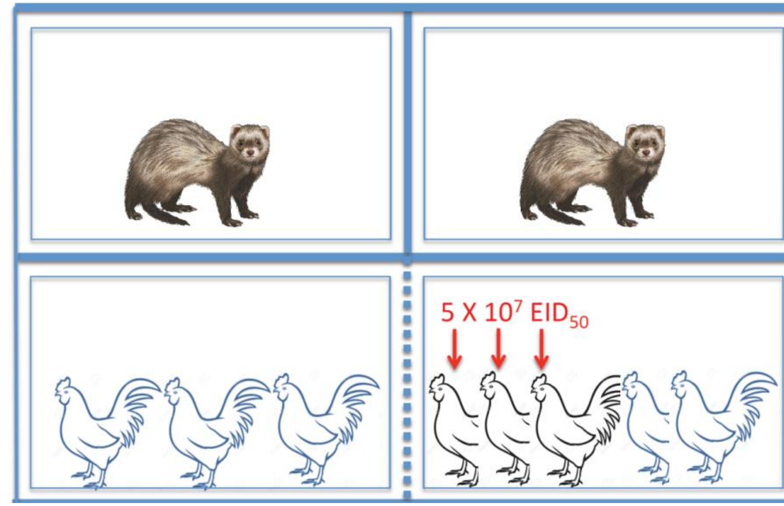
Human and chicken H7N9 isolates showed efficient chicken-to-chicken transmissibility via direct contact

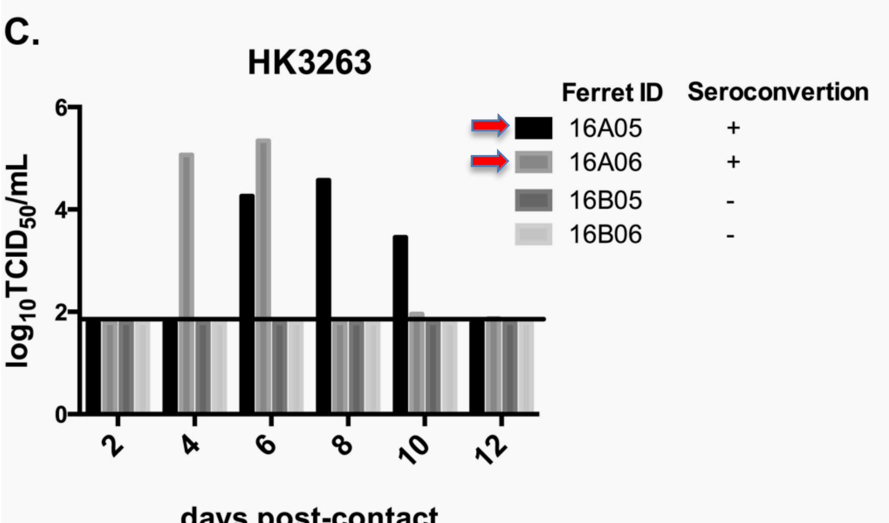


- *Transmission between chickens by direct contact (e.g. sharing water); not by airborne route.*
- *Virus shedding oropharynx >> cloaca*

Chicken-to-ferret airborne transmission was observed for both human and chicken H7N9 viruses







Just as in humans, H7N9 transmission to ferrets is associated with acquisition of PB2 mutations E672K or E627V

Luk et al J Virol 2015

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Case-control study among H7N9 patients (n=89) with age, sex, and neighborhood-matched controls (n=339): *Liu B. et al. CID 2014*

- No poultry contact and not in any location with poultry. Cases 14%; controls 29%).
- In contact with poultry but no direct/indirect contact. Cases 32% vs controls 40% (OR 2.3;).
- Direct or indirect contact. 55% vs. 31% (OR 7.8; 95% CI 3.3-18.8)

All cases 2013-Feb 2017; n=1220
No exposure to poultry: 17%
Wang et al Lancet 2017

- Forgot exposures?
- Another route of exposure?

Contamination of poultry carcasses?

- **Cooking kills influenza viruses.**
- **But possibility of contamination from carcass ?**
  - *Direct contamination of humans from carcass*
  - *Indirect contamination of other foods consumed without cooking?*

Detection of avian influenza virus in chicken carcasses by RT-PCR in live poultry markets in Guangzhou

	Dressed poultry stalls	Retail markets	Super-markets	P value
Oropharyngeal swabs	67/121 (55%)	207/277 (75%)	2/62 (3.2%)	0.01
Cloacal swabs	55/120 (46%)	177/265 (67%)	4/62 (7%)	0.053
Visceral cavity	48/118 (41%)	203/329 (62%)	2/23 (9%)	0.033
% of H7/H5	6%	12%		

Mao X ---- H Yen EID 2017

Detection of avian influenza virus in chicken carcasses by virus culture in live poultry markets in Guangzhou

	Dressed poultry stalls	Retail markets	Super-markets	P value
Oropharyngeal swabs	44/121 (36%)	158/277 (57%)	0/62 (0%)	0.03
Cloacal swabs	38/120 (32%)	133/265 (50%)	0/62 (0%)	0.03
Visceral cavity	23/118 (20%)	93/329 (28%)	0/23 (0%)	0.15

Mao X ---- H Yen EID 2017

Summary

- Separation of aquatic and terrestrial poultry marketing chains can reduce emergence of novel zoonotic avian influenza
- Reported human cases of H7N9 under-estimates extent of human H7N9 infection
- HPAI H7N9 may not have increased virulence for humans, but concern of antiviral (oseltamivir) resistance with HPAI H7N9 disease
- Rest days / banning holding live poultry overnight can reduce viral load in live poultry markets and zoonotic risk
- Avian influenza (including H7N9) can be readily detected in large airborne droplets in vicinity of poultry in live poultry markets → de-feathering machine is a high risk source of virus borne aerosols
- Transmission of H7N9 from chicken to ferret can occur by air-borne droplets → associated by rapid acquisition of mammalian adaptation markers observed in humans.
- Poultry carcasses are contaminated by live avian influenza virus → rate of contamination depends on the rate of virus detection in source poultry → poultry from vertically integrated systems with minimal "pooling" / mixing of poultry have lowest rates of contamination.

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AFCD: Thomas Sit, Geraldine Luk, Chris Brackman, MKW Chow, Elaine Lee et al. (Les Sims, Trevor Ellis).
FEHD: Gloria Tam, Miranda Lee, JF Jai, Candy Ng et al.

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NIAID Centers of Excellence for Influenza Research and Surveillance

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