Why are so few people getting infected in face of large exposed population?

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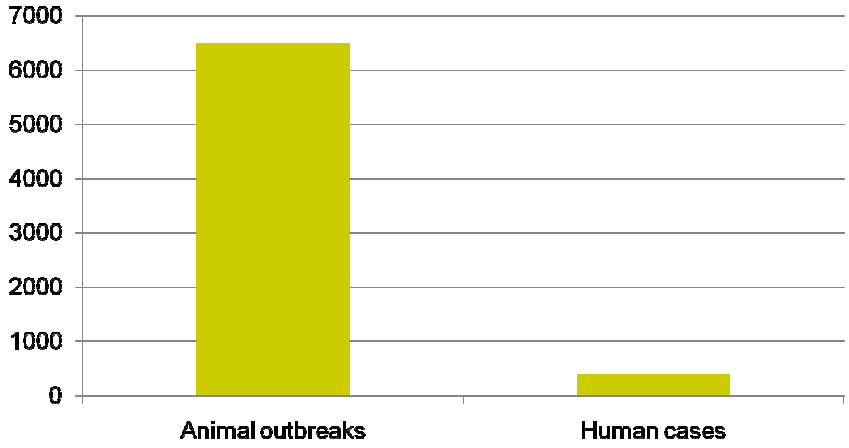
Is premise correct?

Are few people getting infected in the face of large numbers exposed?

Poultry and human cases



Human cases under-recognized?

A patient with suspected avian flu in Asia

Unconfirmed cases in clusters

7/34 = 20%

Ha Nam, VTN
-12y Daughter, Onset: 25/Dec 03
-30y Mother, Onset: 01/Jan 04

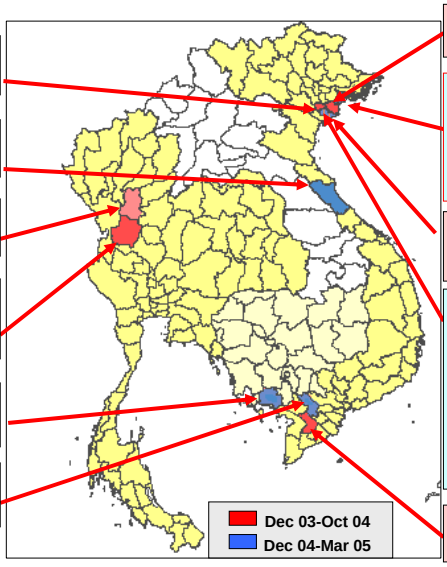
Quang Binh, VTN
-13y Sister died on 9/Mar 05
-5y Brother Admit on 15/Mar,Onset?

Sukhothai, THA
-6y Son, Onset: unknown (04)
-Mother, Onset: same as above

Kamphaeng Phet, THA
-11y Daughter Onset 2/Sep 04
-26y Mother Onset 11/Sep 04
-32y Aunt Onset 16/Sep/04

Kampot ,CAM
-25y Sister Onset: 26/Jan 05
-14y Brother Onset: 20/Jan 05

Dong Thap, VTN
-35y Mother Onset 14/Jan 05
-13y Daughter Onset 20/Jan 05



Nam Dinh, VTN
-7y Sister, Onset: ?
-5y Brother, Onset: 29/Dec 03

Hai Phong, VTN
-35y Father
-33y Mother
-13y Daughter
-10y Daughter,
-4mo Daughter

Thai Binh, VTN
-31y Brother, Onset: 03/Jan 04
-30y Sister, Onset: 10/Jan 04
-23y Sister, Onset: 10/Jan 04

Thai Binh, VTN
-46y Brother, Onset: 5/Jan 05
-42y Brother, Onset: 10/Jan 05
-36y Brother, asymptomatic

-21y Brother, Onset: 14/Feb 05
-14y Sister, Onset: 21/Feb 05
-81y Grandfather
-26y M nurse, Onset: 24/Feb 05

-69y Husband, Onset: 19/Feb 05
-61y Wife, Asymp: 9/Mar 05

Hau Giang, VTN
-19y Brother, Onset: 27/Jul 04
-25y Sister Onset:05/Aug 04

Sub-clinical / mild cases

Hong Kong = 5; Vietnam=3, Turkey=1, Indonesia=3+

Group	Location	Year	Assay	No.	No. +ve
Poultry workers	HK	1997	MN	1525	10%
Poultry cullers	HK	1997	MN	293	9
HCW	HK	1997	MN	528	10
Household contacts	Viet Nam (S)	2004	MN	51	0
Contacts of sick poultry	Viet Nam (S)	2004	MN	25	0
Household contacts	Vietnam (S)	2004	RT-PCR	30	0
HCW with contact	Viet Nam (S)	2004	ELISA, MN	60	0
HCW with contact	Viet Nam (N)	2003-4	MN, WB	83	0
HCW with contact	Thailand	2004	Clinical only	54	0
HCW with contact	Thailand	2004	MN (paired sera)	25	0

Group	Location	Year	Assay	No.	No. +ve
HCW with contact	Thailand	2004	Clinical only	35	0
Poultry cullers	Japan	2004	MN	58	5 (8.6%)
Poultry cullers	Indonesia	2005	MN	79	1
Contacts of cases	Indonesia	2005	MN	170+	0
Villagers ≤ 1 km of fatal human A(H5N1) infection in area with poultry outbreaks	Cambodia	2005	MN, WB	351	0
Close contacts of cases	Cambodia	2005-6	Clinical, RT-PCR, MN	80	0
Wet poultry market workers	China	2006	MN	110	1
Adults with CAP in ICU	Thailand	2005-6	TA for RT-PCR, virus culture; MN, IF	115	0
Close household or social contacts of patients HCW contacts of H5 patients Poultry exposures	China	2005-7	Clinical + RT-PCR and paired serology (MN, HAI) if ill	641 745 523	0 0 0
villagers	Cambodia	2006	MN + Western Blot	674	7

Conclusion

1. There are more severe cases than reported
2. There are sub-clinical cases - but unlikely to be large numbers
3. Never the less, there are a small number of cases given the large number of people exposed
4. There is a substantial “barrier to transmission”

Gaps & actions

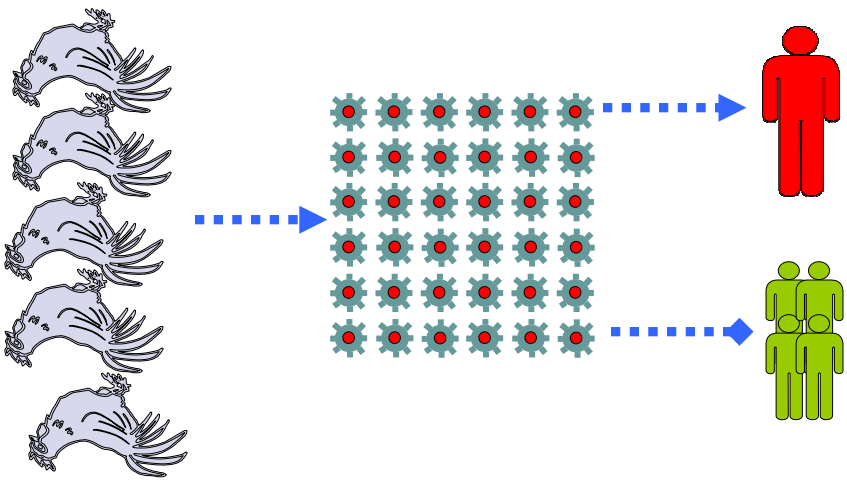
- Interpretation of serosurveys
- Estimate of true case incidence
- Estimate of numbers exposed
- Estimate of risk
- Study kinetics of antibody responses in survivors & controls
- Systematic review & meta-analysis with estimates of uncertainty
- Estimate of incidence + estimate of exposure = estimate risk

Why we need a 'risk assessment'

- Risk reduction – honest education
- Advocacy
- Resource allocation
- Base-line for surveillance & monitoring
- Base-line for evaluation
- Identify risk groups (familial relative risk)
- Guide science (e.g. sample size)

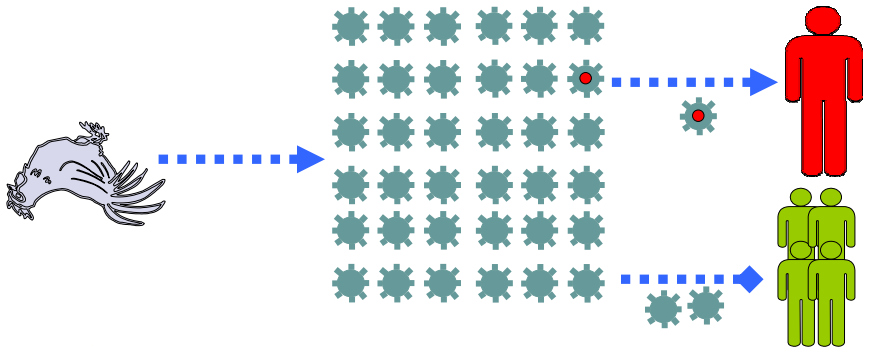
Why so few cases?

What are barriers to transmission?



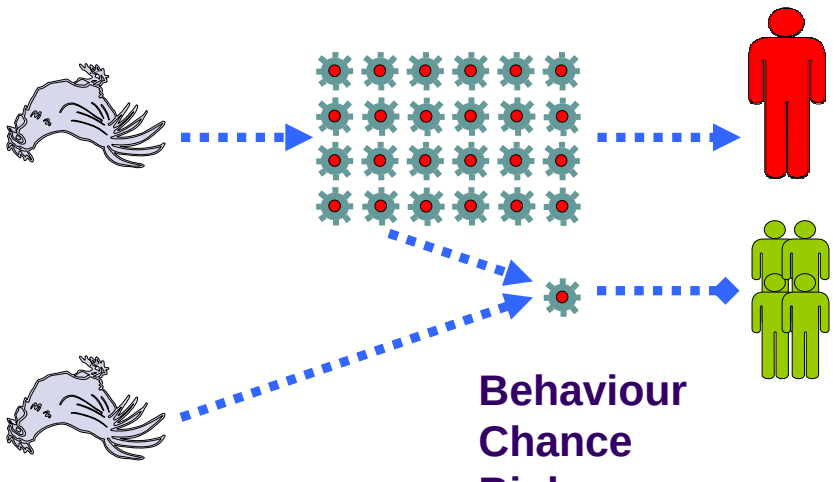
Virus?

Yes...but role of heterogeneity?



Variations
– binding, replication, immune stimulation

Dose / exposure intensity?

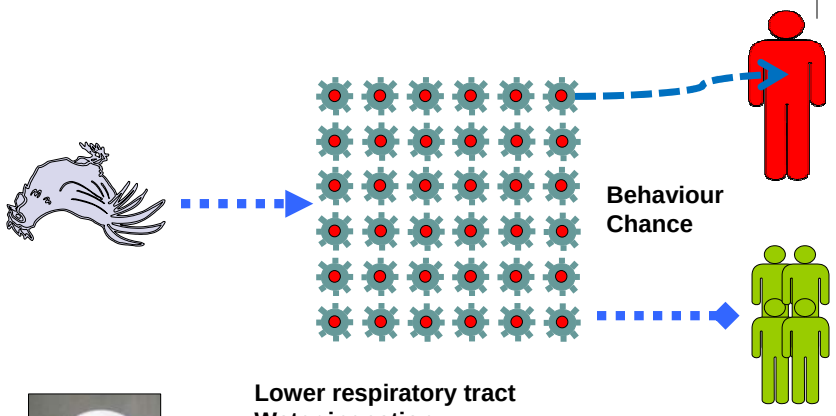


Behaviour
Chance
Biology

But.....

- Cullers exposed to high doses
- Many people exposed to '*sufficient dose*'
  - Birds excrete a lot of virus
  - Environmental contamination widespread
- 30% of cases do not report any poultry exposure
- 25% of controls report high risk exposure

Site of inoculation?

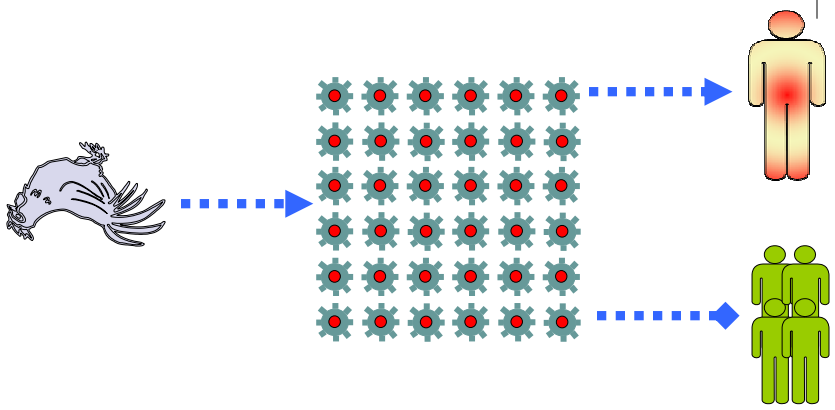


Lower respiratory tract
Water ingestion
Water on conjunctiva
Food ingestion
(poultry products, contaminated vegetables)

But.....

- Case-control studies have generally not indentified important ‘unusual exposures’
- Attributable risk of ‘unusual exposures’?
  - Swimming in rivers & lakes
  - Raw ducks blood
- Direct contact with sick birds is main risk
- A common risk:
  - Cullers
  - 25% of controls report high risk exposure
  - Population attributable risk percent is only 28%
- 30% of cases do not report any poultry exposure

The victim?



Host heterogeneity

Host factors

- Immunity? – antibodies or CMI
  - *Age profile*
  - *Cullers*
- Intrinsic susceptibility?
  - Binding
  - Replication
  - Immune response
  - Release
  - Genotypic
  - Phenotypic – co-factors (age, illness...)

Genetic susceptibility?

Some features of H5N1 epidemiology suggest a host genetic influence on susceptibility to infection:

•Family clustering of cases (*bias?)*

•Few non-related clusters

•Cases in related people separated by time and distance

•Person to person transmission amongst relatives (not unrelated contacts & HCWs)

Family clusters of H5N1 cases

Country	Total cases	No. of family clusters*	n/N (%) of confirmed cases occurring in family clusters
Vietnam	101	12	24/101 (24)
Thailand	25	3	5/25 (20)
Cambodia	7	1	1/7 (14)
Indonesia	116	14	30/116 (26)
China	27	2	3/27 (11)
Turkey	12	3	8/12 (67)
Azerbaijan	8	2	7/8 (87)
Egypt	43	2	5/43 (5)
Iraq	3	1	2/3 (67)
Pakistan	1	1	1/1 (100)
Total	**348**	**41**	**86/348 (25)**

*Cluster defined as at least two cases of clinically compatible illness with at least one case laboratory confirmed H5N1.

Indonesia

- Karo cluster
- Siblings affected six months apart

Multi-generation H2H in families

- Karo
- Pakistan

Conclusions

- Viral factors - ✓
  - but not whole story – does not explain patterns
- Dose / exposure intensity - X
- Site of inoculation / behaviour – ?
- Host factors - ????

Gaps

- Host factors

Recommendations

- Look at host phenotype
  - Antibody and CMI (?)
  - Receptors
  - Gene expression (mild vs severe H5 vs seasonal)
- Look for evidence of genetic effect
  - Increased familial relative risk
  - Genetic studies

All do-able !


optimism:
a new way of life
Optimism is the key to a joyful, successful and fulfilling life, recommends Bob Farrant.

Design and methods

- Conduct a **linkage study** in families with multiple H5N1 cases to investigate if any single nucleotide polymorphisms (SNPs) are transmitted to affected offspring more or less often than expected;
- Conduct a genome-wide, **case-control association study** to investigate if any SNPs occur at a significantly different frequency in affected individuals compared to unaffected individuals.

Linkage study

- Use related individuals
- To identify regions of genome that contain genes predisposing to disease
- By looking for marker loci transmitted from parents to affected offspring more often than expected.
- Since loci close to each other on the same chromosome are more likely to be inherited together than loci far apart
- The marker loci should be located near the gene predisposing to disease

Linkage study

- Assemble pedigrees of multiply affected families
- Ascertain availability of DNA
- Obtain consent and specimens
- Genotype with genome-wide linkage panel of 10,000 SNPs
- Undertake linkage analysis, looking for regions of the human genome inherited with the disease state by calculating parametric and non-parametric logarithm of the odds (LOD) scores.

Association study

- Use unrelated individuals
- To identify regions of genome that contain genes predisposing to disease
- By looking at the frequency of genetic markers in cases and controls
- Markers that appear more frequently in cases compared to controls may be located near the gene predisposing to disease

Association study

- Identify suitable cases
- Proceed if sufficient cases can be enrolled
- Identify suitable control groups
  - For Vietnam – community cohort or poultry cullers or cord blood controls
- Genotype using a genome-wide genotyping kit of more than 550,000 SNP loci
- Calculate odds ratio, chi-square test statistic, Armitage trend and p values for the association between individual SNP genotype and disease status

Genome-wide not candidate gene approach

Few data on genetic determinants of influenza infection and disease.

Possible candidate genes include:

- Cell surface sialic acids recognised by viral haemagglutinin
- MX1 gene (interferon induced factor)
- Toll like receptors

But little solid data to support any choice.