



Columbia University
MAILMAN SCHOOL
OF PUBLIC HEALTH

One Health: Emerging Infections and Biosafety Challenges

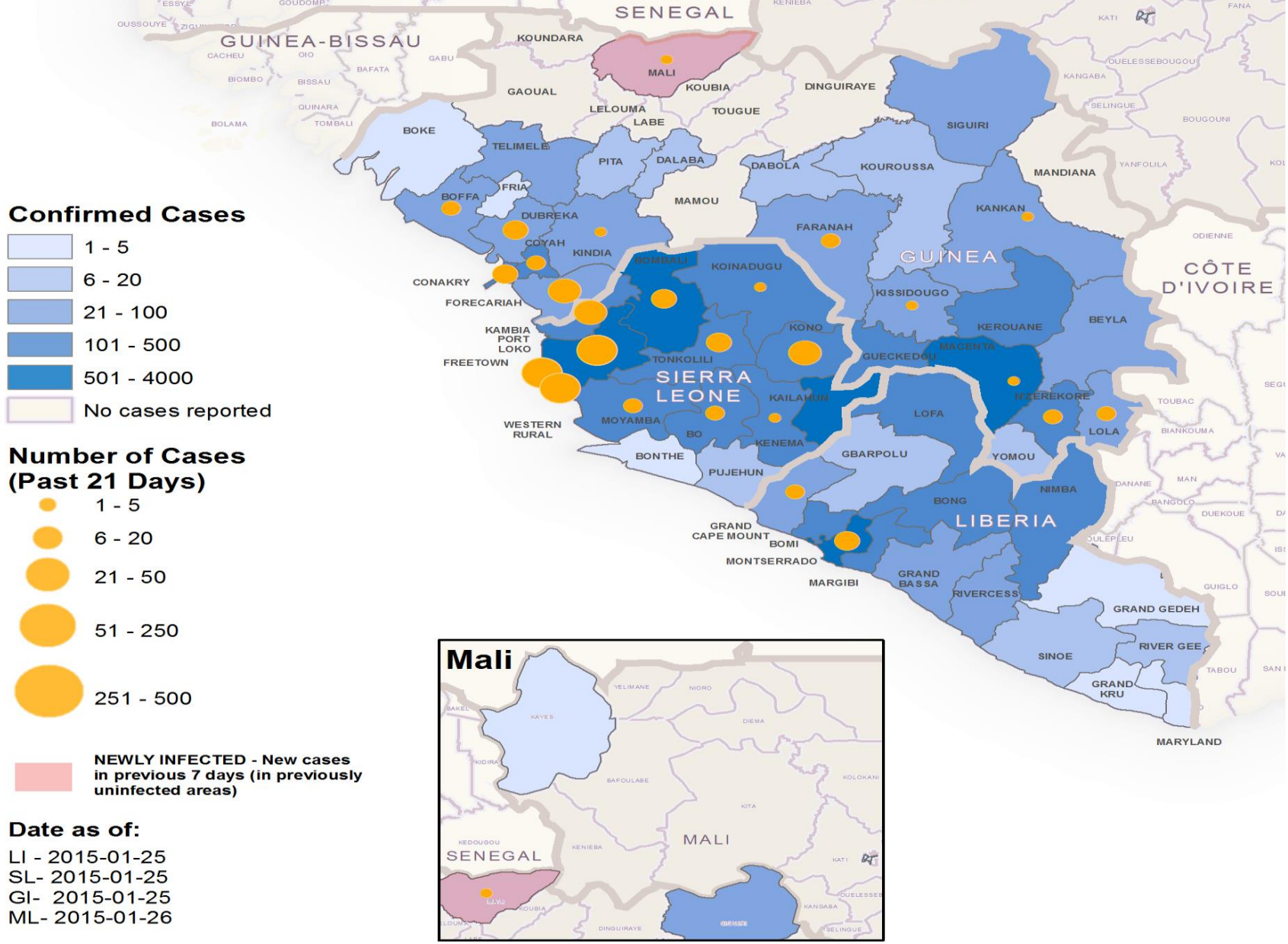
Stephen S. Morse

Third International USDA/ABSA Biosafety and
Biocontainment Symposium:
Biorisk Management in a One Health World

Current Outbreaks of International Public Health Concern

	Main location, date outbreak started	Cases (deaths)
H5N1 avian influenza	Asia (now 16 countries worldwide), since 2003 (orig. 1997)	719 (413 deaths)
H7N9 avian influenza	China, March 2013	541 (33) [Unofficial]
MERS [Middle East Respiratory Syndrome] Coronavirus	Middle East Gulf States, since April 2012	956 (\geq 351)
Ebola	West Africa (Guinea, Liberia, Sierra Leone)	22,334 (8,921 deaths)

Source: WHO and other sources



Ebola Virus Disease in West Africa

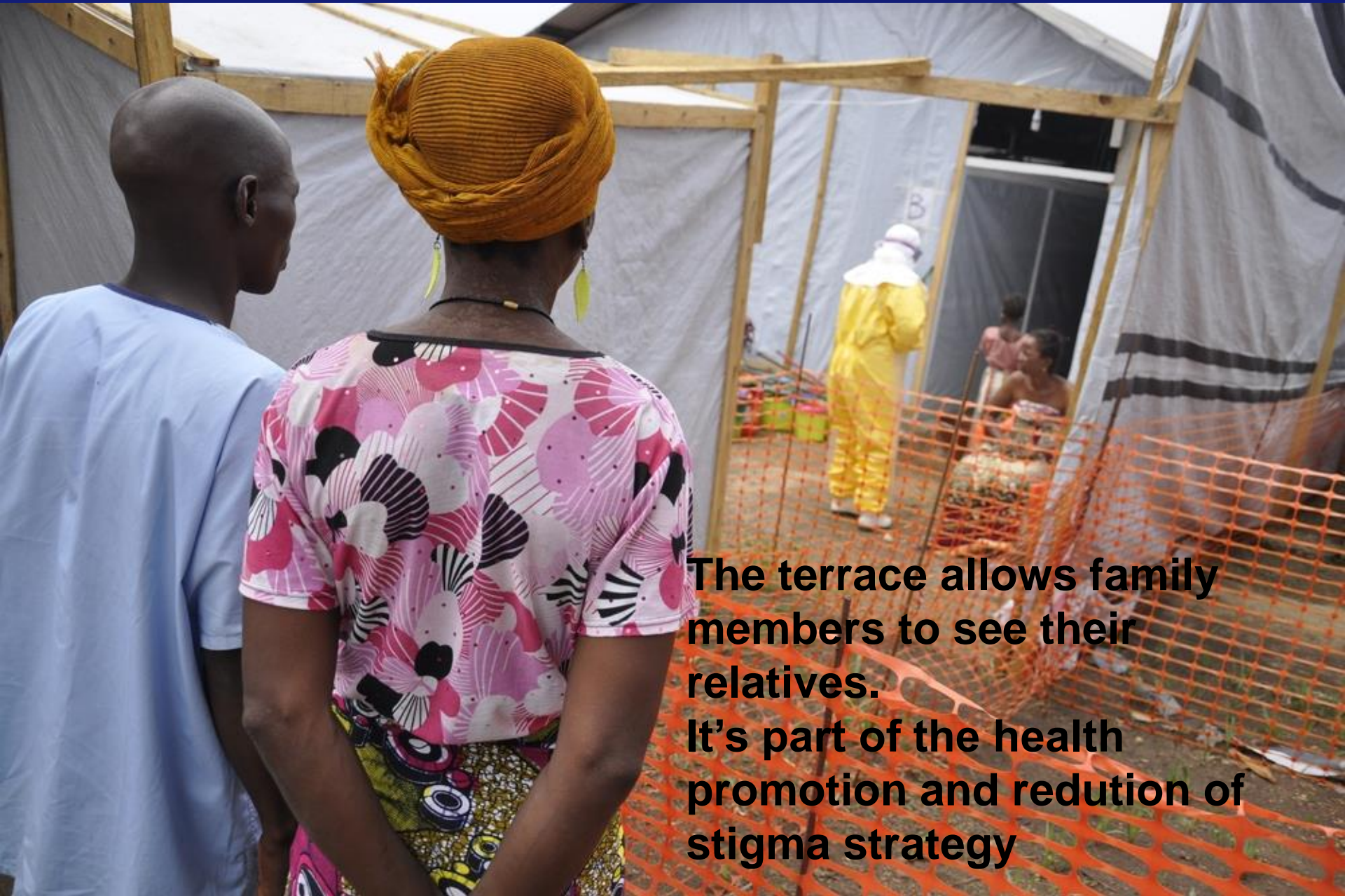
Small but Deadly



RNA genome

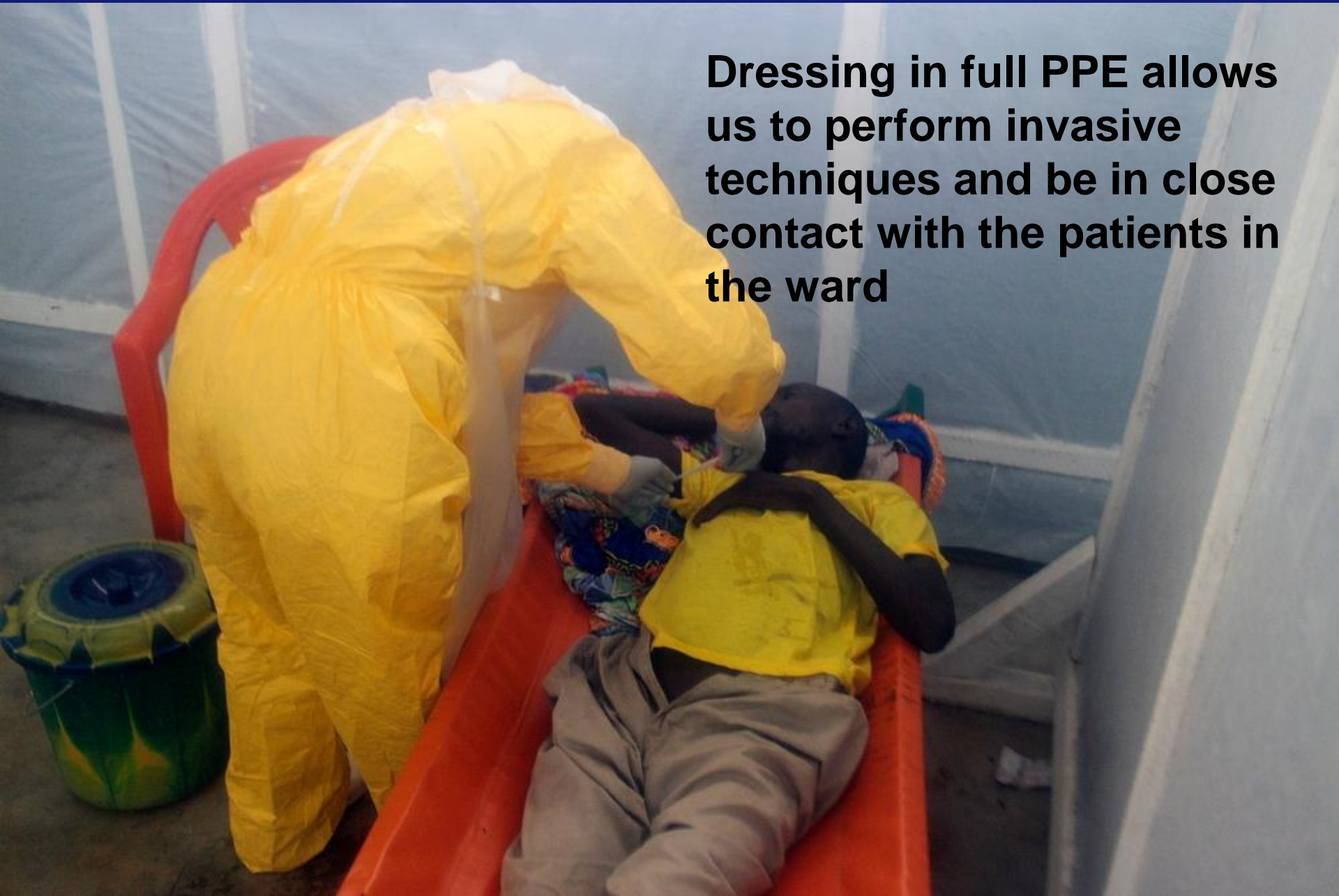
Enveloped (membrane coated)

Inactivated by heat, solvents, most disinfectants



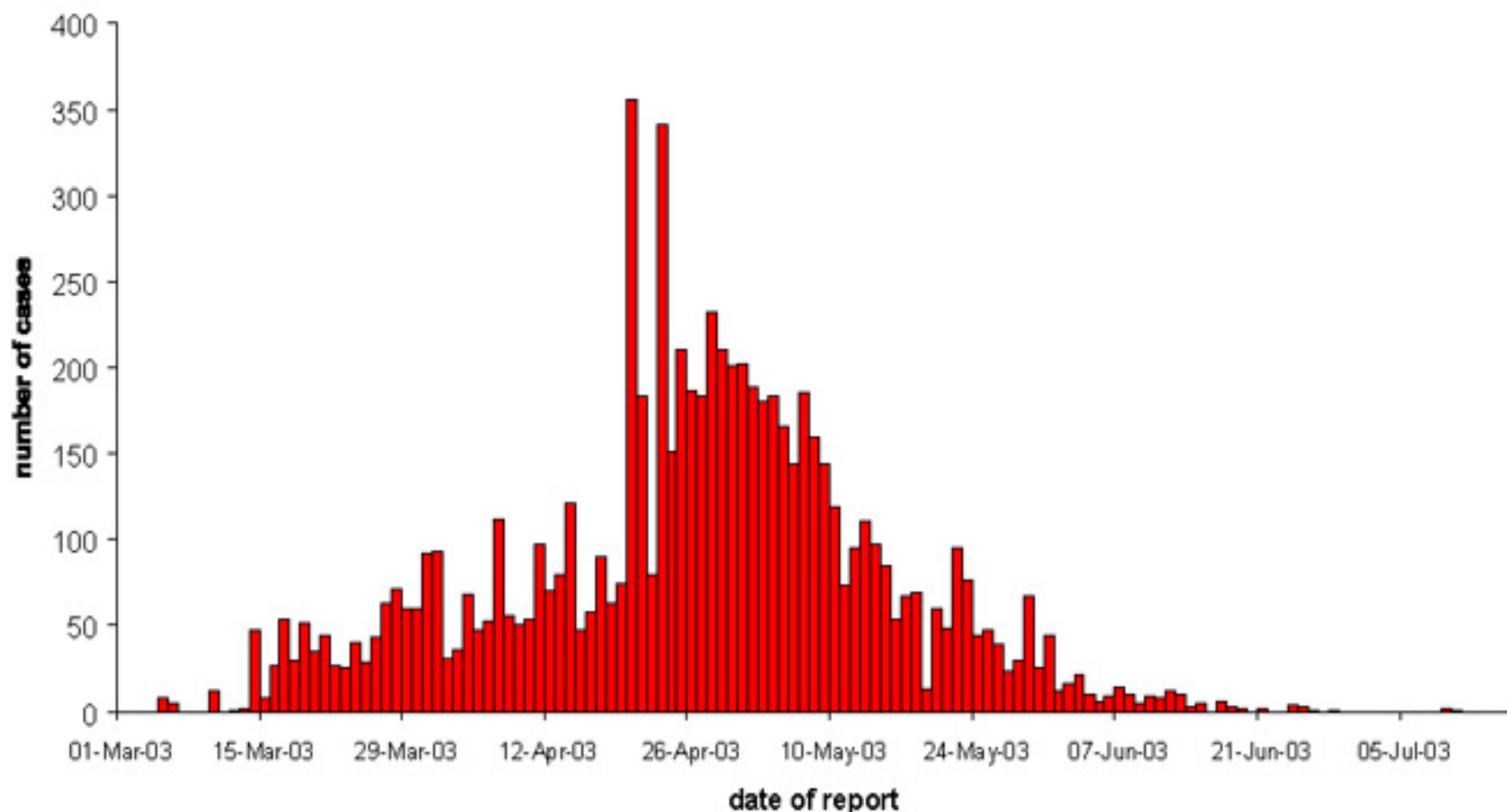
The terrace allows family members to see their relatives. It's part of the health promotion and reduction of stigma strategy

Dressing in full PPE allows us to perform invasive techniques and be in close contact with the patients in the ward





**Probable cases of SARS by date of report
Worldwide* (n=7,588), 1 March - 10 July 2003**



* As of 10 July 2003, 8,437 probable cases of SARS have been reported to WHO. This graph includes all cases from Hong Kong SAR, Macao SAR and Taiwan, China, but only those cases elsewhere in China reported after 3 April 2003 (1,190 cases between 16 November 2002 and 3 April 2003 not shown). Also includes 341 probable cases of SARS who have been discarded and for whom dates of report could not be identified. The United States of America began reporting probable cases of SARS to WHO on 20 April 2003

SARS and the economy: impact on global travel, Hong Kong



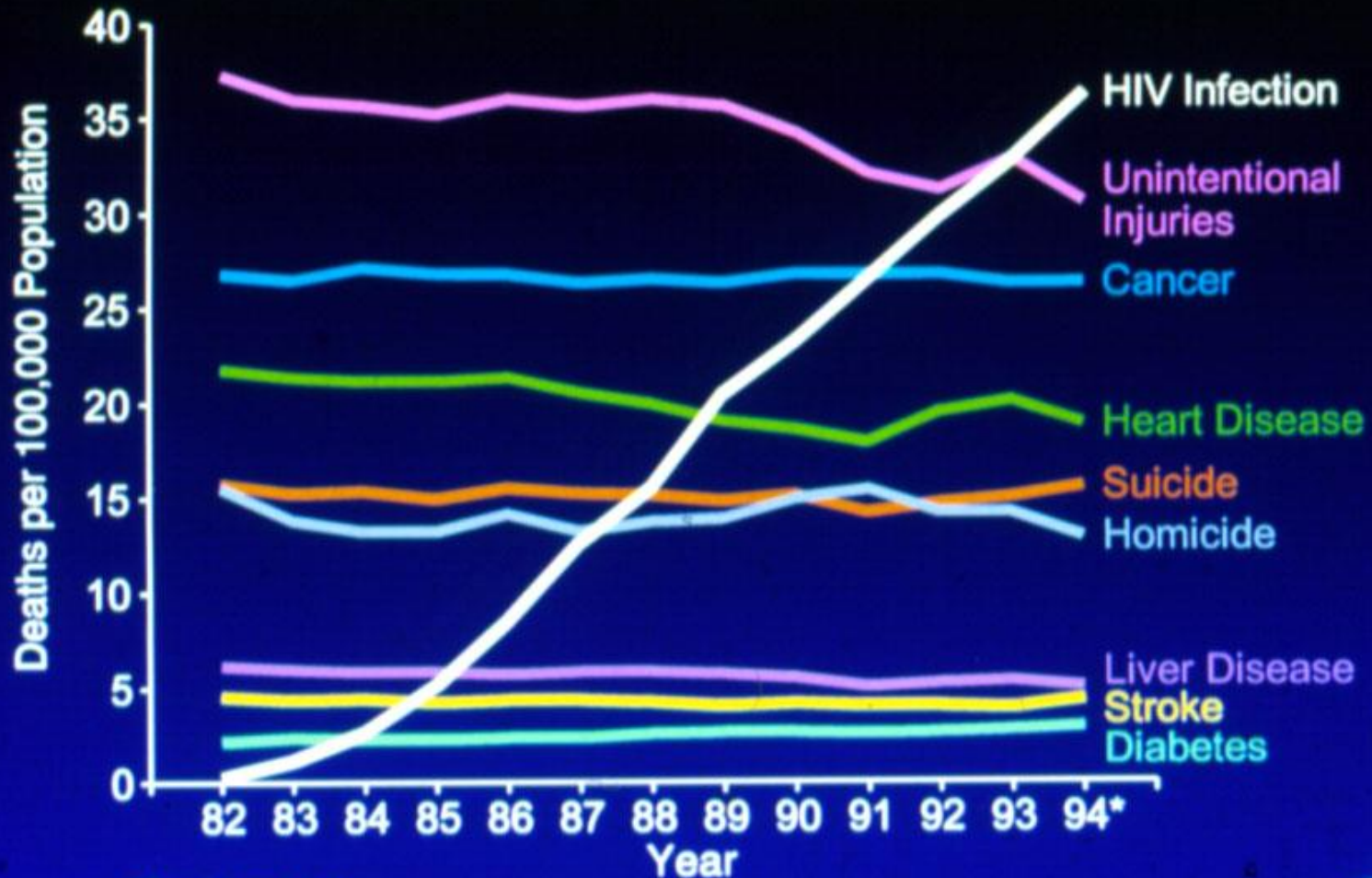
Slide courtesy of Dr. Isaac Weisfuse, NYC DOHMH

THE NEW YORKER





Death Rates from Leading Causes of Death in Persons Aged 25-44 Years, USA, 1982-1994



*Provisional data

SOURCE: National Vital Statistics

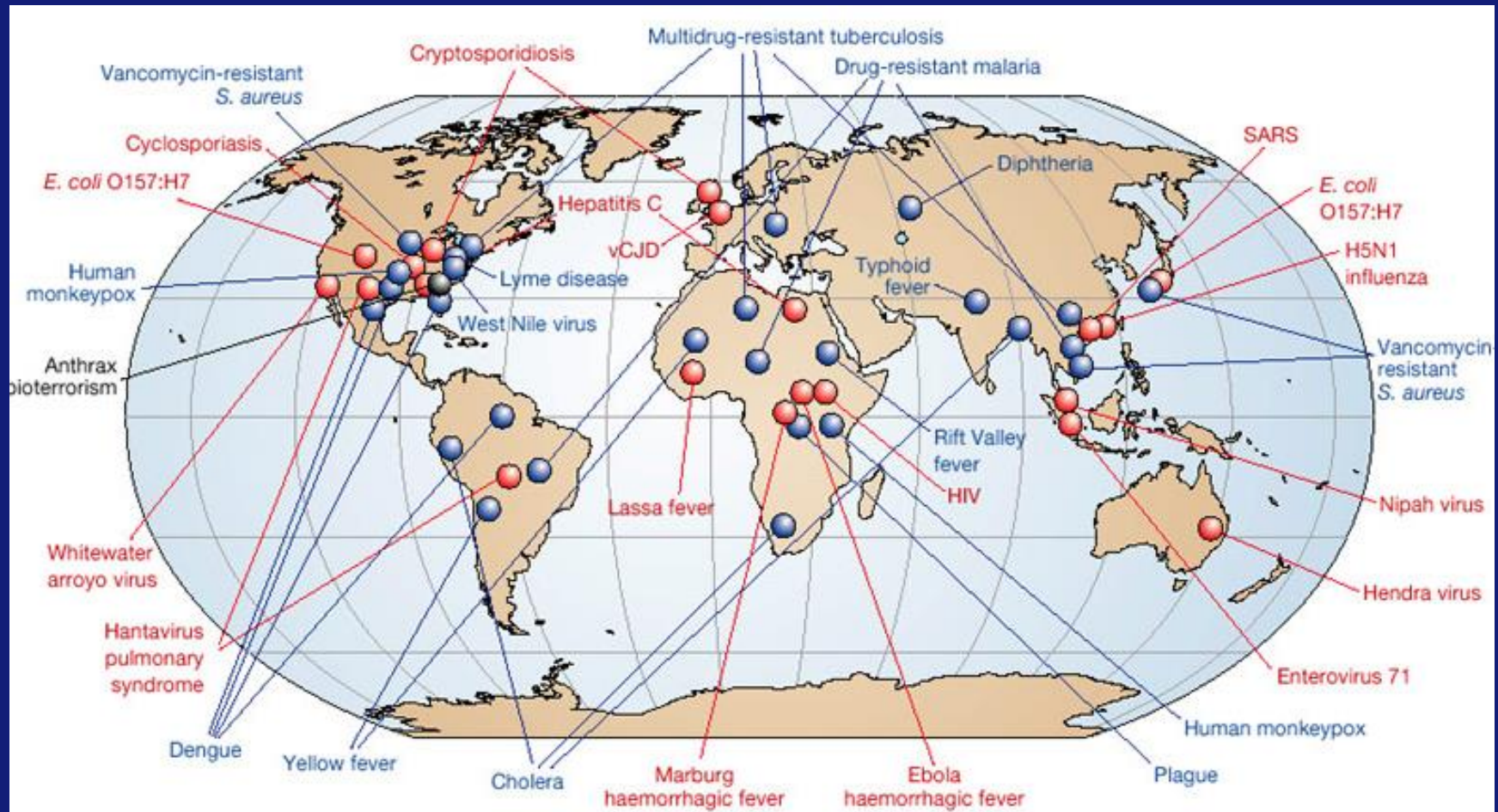
Emerging Infections

- **Those rapidly increasing in incidence (number of new cases) or geographic range**
- **Often novel (a previously unrecognized disease)**
- **Anthropogenic causes often important in emergence**

EMERGING INFECTIONS: SOME RECENT EXAMPLES

- **Ebola, 1976 –**
- **HIV/AIDS**
- **BSE & Variant CJD, ca. 1986 –**
- **Hantavirus pulmonary syndrome, 1993**
- **Hemolytic uremic syndrome, 1990's –**
- **Nipah, 1998 –**
- **West Nile, US, multistate, 1999 –**
- **SARS 2003 – (and MERS-CoV 2012 –)**
- **Influenza (including H5 in Asia 2003 –;
H1N1 pandemic 2009-10; H7N9 avian flu,
China, 2013–)**

Global Examples of Emerging and Re-Emerging Infectious Diseases



Courtesy NIAID (Dr. Anthony Fauci)

Zoonoses in disease emergence

- 1407 human pathogens
- 58% are zoonotic
- 130 of the 177 recently emerged pathogens are zoonotic (RR=2.0)
- Only a few transmit human-to-human

--[Numbers from: Woolhouse ME, Gowtage-Sequeria S. *Emerg Infect Dis* 2005; 11(12): 1842-7.]

Courtesy Dr. Larry Madoff

New Opportunities for Pathogens: Ecological Changes

Agriculture	Hantaan, Argentine Hemorrhagic Fever, Nipah, West Nile (Israel), possibly pandemic influenza
Food handling practices	SARS, H5N1 influenza, HIV?, Enteropathogenic <i>E. coli</i>
Dams, changes in water ecosystems	Rift Valley Fever, other vector borne diseases, Schistosomiasis
Deforestation, reforestation	Kyasanur Forest, Lyme disease
Climate changes	HPS, vector borne diseases

Amplifiers for Emerging Infections

- Healthcare settings (infection control)
- Live animal markets and food handling
- Hunting

Why is “One Health” Important?

- **Most emerging infections are zoonotic**
 - **crossing species**
- **Thus, many of the emerging infections of the future can be found in other animal species**
- **Humans may become infected through:**
 - **Changes in environment that increase contact (wildlife)**
 - **Handling of food animals**
- **Therefore, surveillance across species is essential**

Caution
Wildlife Can Be
Dangerous



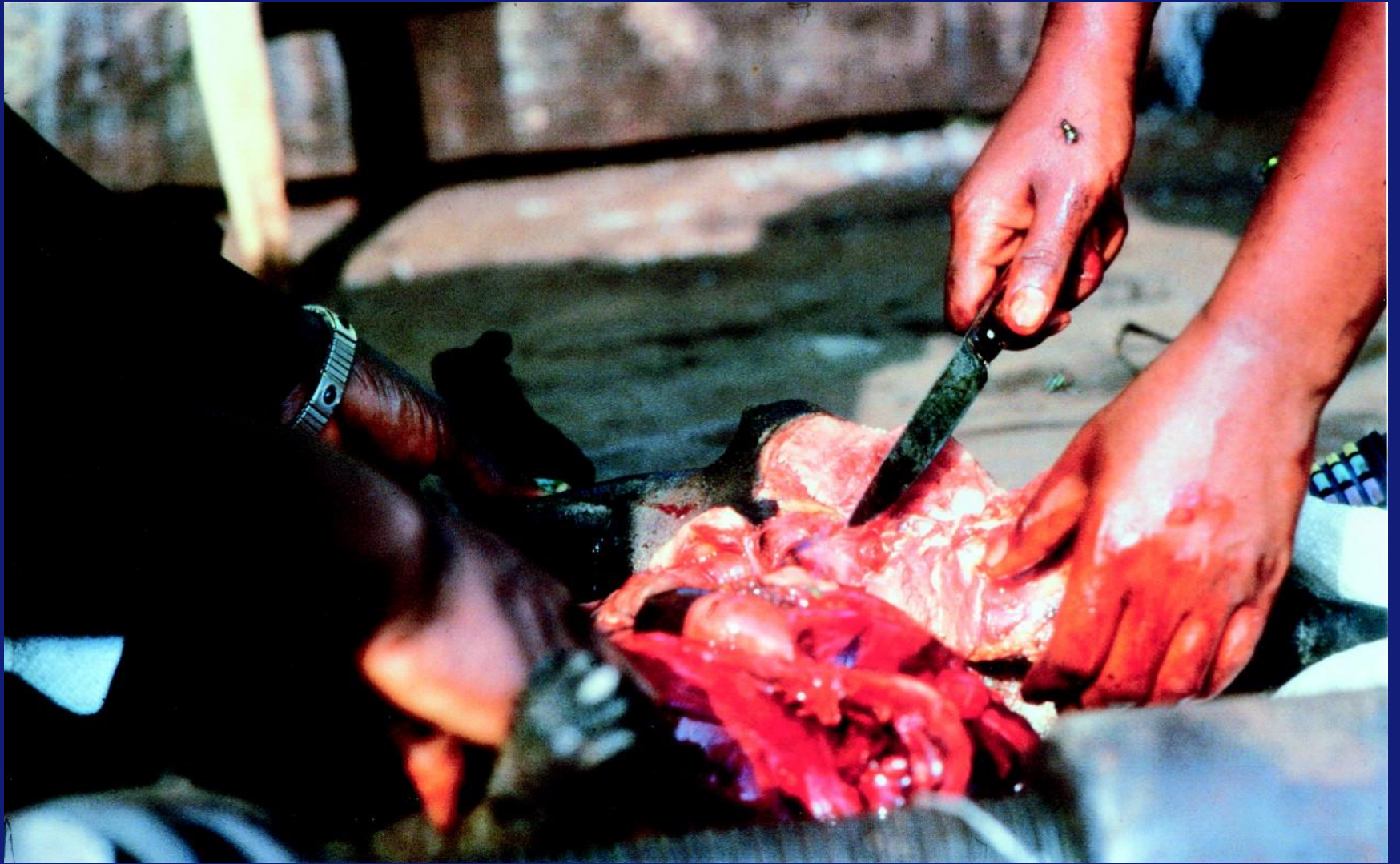
Wildlife/livestock contact



Hunting Markets/trade



Courtesy Dr. William Karesh (market in Jakarta, Indonesia)



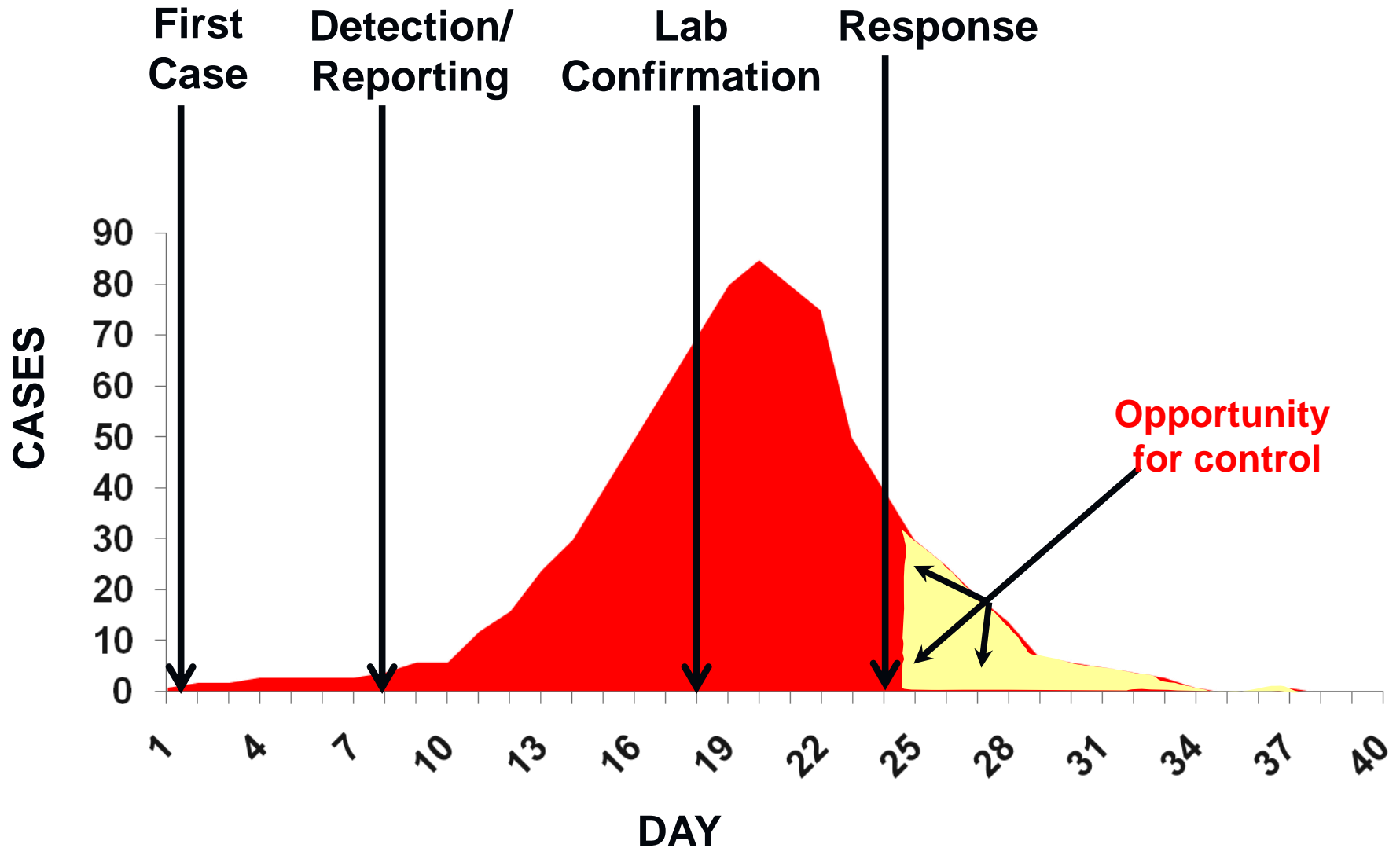
Photograph: Karl Ammann; from Hahn *et al.*, 2000

No pandemic or emerging
infection has ever been
predicted

--- Morse *et al.*, Lancet 380: 1956–1965 (2012).

Every expert group studying this
issue has recommended
**Effective global surveillance
and early warning
as the first priority**

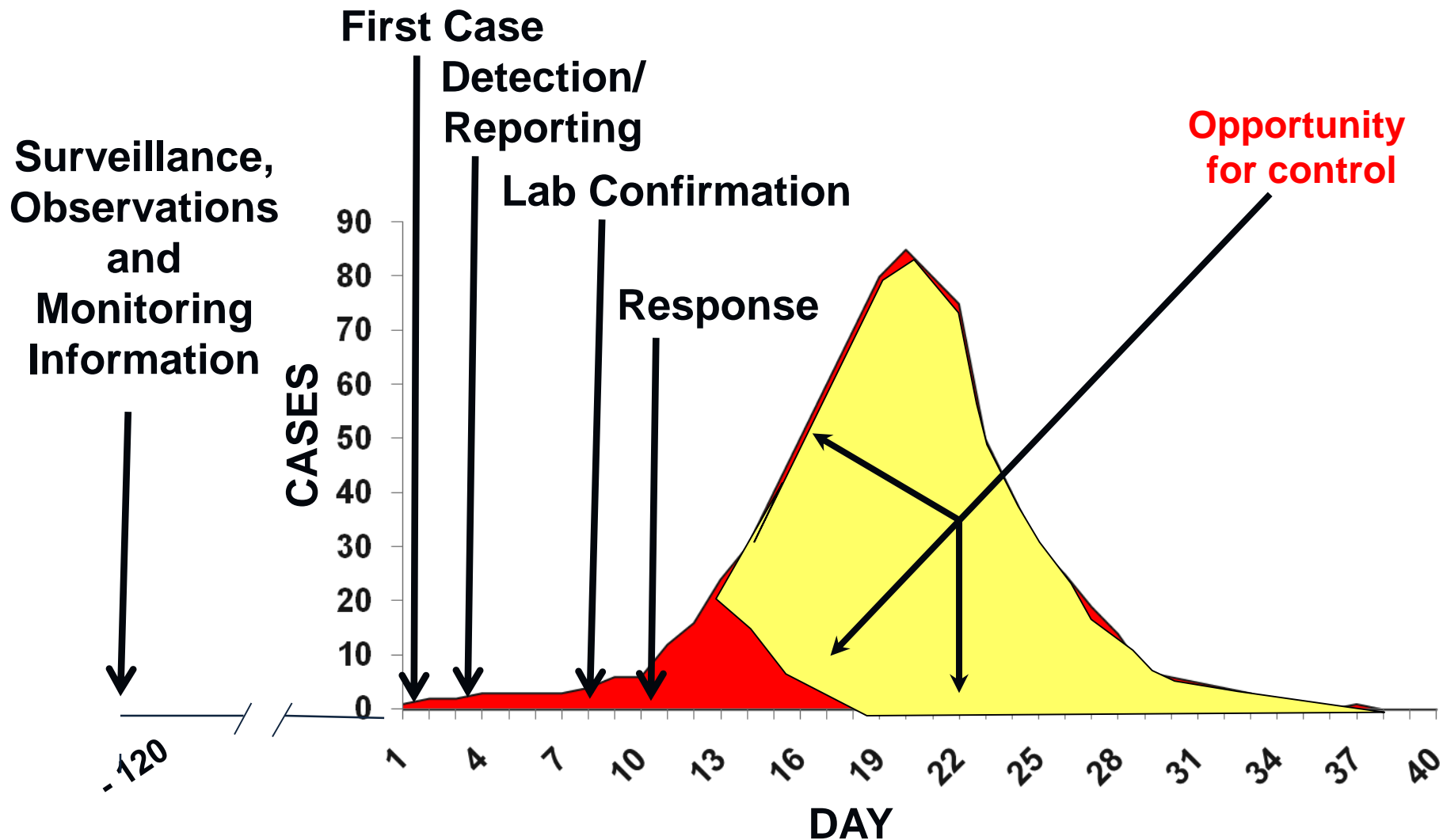
Current Outbreak Detection and Response



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PREDICT

Effective Health Early Warning



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FROM THE AMERICAN PEOPLE

PREDICT

Gaps in Disease Early Warning Systems

- Priorities vary among different jurisdictions
- Many gaps in chain of communications
 - Real situation often does not reflect org chart
- Most reporting voluntary, often low priority
- Government embarrassment over adverse information
- Fear of adverse effects

How Can We Get Earlier Global Warning?: Some Attempts at Improvement

ProMED-mail: A Prototype Outbreak Reporting System

ProMED-mail:

www.promedmail.org

- Moderated listserv
- Free to all
- Started 1994
- Approximately 60,000 subscribers in \geq 185 countries


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Latest Posts on ProMED-mail

- 05 Sep 2012 West Nile virus - USA (12): (TX, OK)
- 05 Sep 2012 Yellow fever - South America (07): Bolivia (BE)
- 05 Sep 2012 Epizootic hemorrhagic disease, cervids - USA (12): (IN, MI)
- 05 Sep 2012 Hepatitis A - France, Philippines: food establishments susp.
- 05 Sep 2012 Human enterovirus 71 - Cambodia (02): genotype C4 sequenced
- 05 Sep 2012 E. coli EHEC - USA (28): (MI) prison
- 05 Sep 2012 Ebola virus disease - Uganda (23): (KI) new susp. case
- 05 Sep 2012 Dengue - Greece (WG) susp.
- 05 Sep 2012 West Nile virus - Eurasia (06): Serbia
- 05 Sep 2012 Leptospirosis - Philippines (03): (PN) fatal, flooding
- 04 Sep 2012 Ebola virus disease - Congo DR (08): (OE)
- 04 Sep 2012 Announcements (04): OIE, WAHIS, new version
- 04 Sep 2012 Eastern equine encephalitis - USA (15): (VT), human
- 04 Sep 2012 Echinococcus multilocularis, canine - Canada: (BC)
- 03 Sep 2012 Trypanosomiasis, foodborne - Brazil (PA)
- 03 Sep 2012 Ebola virus disease - Uganda (22): (KI) WHO update
- 03 Sep 2012 Crimean-Congo hem. fever - Pakistan (05): (PB) fatal case

ProMED-mail alerts on HealthMap



Most Recent Alert

 [View printable version](#) Share this post:    

Published Date: 2012-09-05 21:06:05

Subject: PRO/AH/EDR> West Nile virus - USA (12): (TX, OK)

Archive Number: 20120905.1282592

WEST NILE VIRUS - USA (12): (TEXAS, OKLAHOMA)

A ProMED-mail post

<http://www.promedmail.org>

ProMED-mail is a program of the International Society for Infectious Diseases <http://www.isid.org>

In this report:

[1] Texas

[2] Oklahoma

[1] Texas

Date: Tue 4 Sep 2012

Source: Statesman [edited]

<http://www.statesman.com/news/local/austin-man-dies-from-west-nile-virus-numbers-2450727.html>

West Nile virus illnesses in Texas continue to rise dramatically, state health officials said Tuesday [4 Sep 2012], with the number of cases this summer rising to 1013 -- with 40 deaths -- as an Austin man became the 2nd in Travis County to die from the mosquito-borne disease [virus].

A little less than 2 weeks ago, there were 640 cases and 23 confirmed deaths statewide. That is a 58 percent increase in cases and 74 percent increase in deaths. State officials warned that the infections may continue until the 1st hard freeze of the year.

"The peak for West Nile season is August, and then there is a delay before it gets reported to us. We are expecting the numbers to keep increasing," said Christine Mann, a spokeswoman for the Texas Department of State Health Services. The agency's count does not include the new Travis County death, which had not yet been reported to the state. As of Tuesday [4 Sep 2012], there have been 48 confirmed cases of West Nile in Travis county, up from 23 cases and one death on 22 Aug [2012].

A Compliment?

"The popular ProMED-mail e-list offers a daily update on all the known disease outbreaks flaring up around the world, which surely makes it the most terrifying news source known to man."

– *Steven Johnson*

"The Ghost Map", p. 219

Riverhead Books/Penguin, 2006

PREDICT Surveillance

Activities of Interest:

- Hunting
- Markets/trade
- Wildlife/livestock conflict
- Morbidity/mortality events
- Free-ranging – undisturbed
- Logging/deforestation
- Water restriction



USAID
FROM THE AMERICAN PEOPLE

PREDICT

The good news:

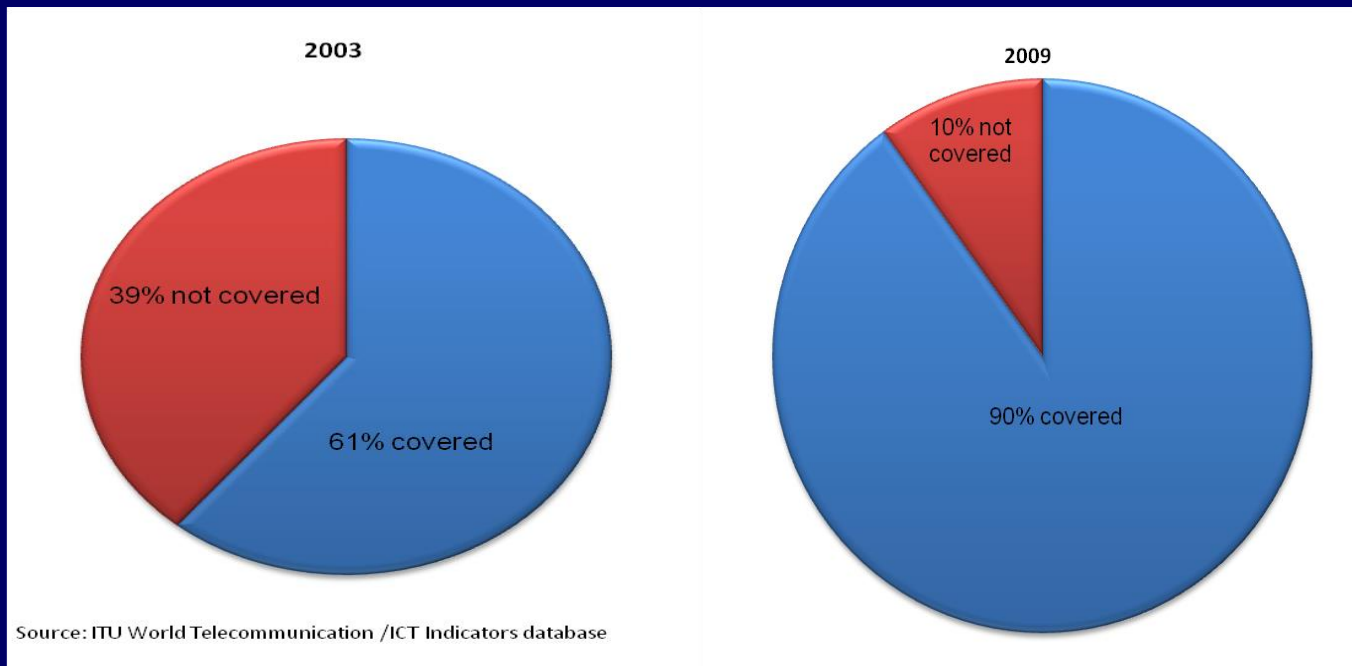
**New technologies in
diagnostics and
communications have
revolutionized ability to
identify and report infections**

**We have gone from a paucity
of data to a flood of data**



Dr. Jonna Mazet, UC-Davis

Percentage of the World's Population Covered by a Mobile Cellular Signal, 2003 vs. 2009



Source: ITU World Telecommunication/ICT Indicators database

NEW. MORE READS. LONGER READ LENGTHS.



More sequencing capacity than ever before.

The industry's most accurate and easiest-to-use benchtop sequencer just got better. [New MiSeq reagents](#) enable up to 15 Gb of output with 25 M sequencing reads and 2x300 bp read lengths. Access even more applications such as exome, mRNA sequencing, targeted gene expression, metagenomics and HLA typing. [See the proof](#) »



Exome



RNA-Seq



Custom Amplicon



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Tracking H7N9 Influenza in China

Read how dedicated researchers at the Jiangsu CDC use MiSeq for infectious disease surveillance.

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Explore MiSeq Speed and Performance

Up to 15 Gb and 2 × 300 bp runs—with the highest data quality.

[See the data](#) »

Generate More Data, with Higher Quality

Dr. Tim Stinear discusses how MiSeq provides higher quality scores and greater output than Ion Torrent PGM.

[See the Results](#) »

Simplify Sample Prep

Explore the industry's fastest—and easiest—sequencing workflow.

[View the application note](#) »

How are people preparing samples?
[View the chart](#) »

Get MiSeq Updates

Interested in receiving newsletters, case studies, and information on new applications? Enter your email address below.

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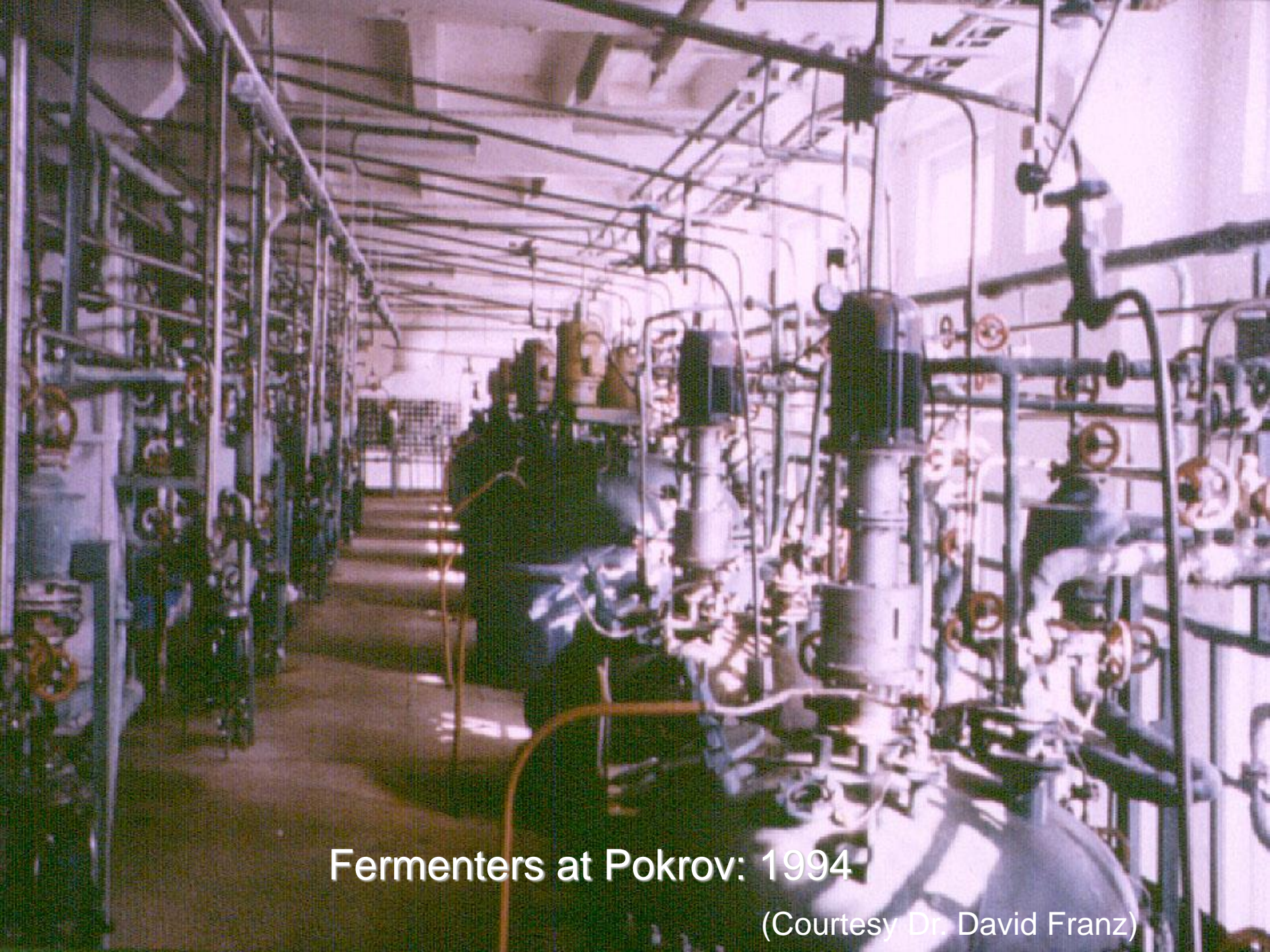
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SIGN UP



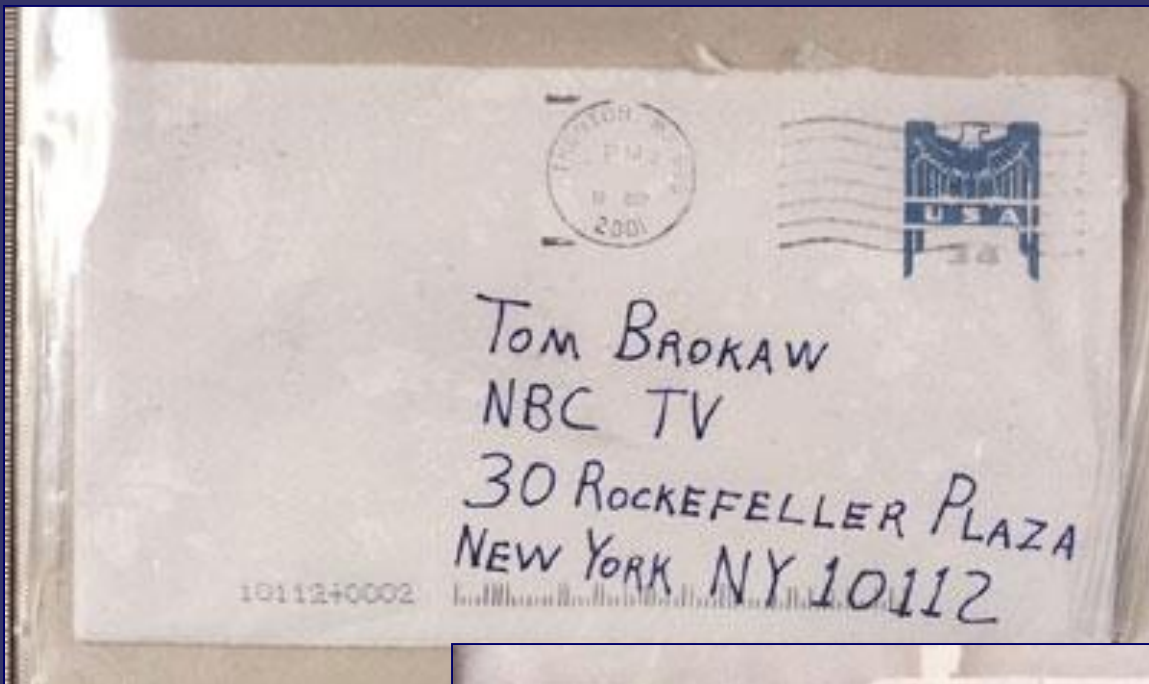


Obolensk: 1994
(Courtesy Dr. David Franz)



Fermenters at Pokrov: 1994

(Courtesy Dr. David Franz)



The “Dual Use” Dilemma

- *Life sciences research underpins:*
 - Biomedical and public health advances
 - Improvements in agriculture
 - Safety and quality of food supply
 - Environmental quality
 - Strong national security and economy
- *However, good science can be put to bad uses*



What Novelties Were Feasible By 2001?:

Existing Examples

- Anthrax modified with gene insert from a non-pathogenic relative can defeat live anthrax vaccine
 - Pomerantsev *et al.*, *Vaccine* 15(17-18):1846-50 (1997).
- Multi-drug resistant anthrax (vaccine strain)
 - Pomerantsev *et al.*, Intl. Anthrax Meeting, Salisbury, 1995.
- Poxviruses with IL-4 gene insert can cause severe disease in immunized or genetically resistant animals
 - Jackson *et al.*, *J. Virol.* 75:1205-1210 (2001);
 - Buller *et al.*, *Applied Biosafety*, 2004.
- Reconstruction of viable 1918 pandemic influenza virus
 - Tumpey *et al.*, *Science* 310:77-80 (2005).



Some Efforts to Define the Biothreat Problem

- *“Biotechnology Research in an Age of Terrorism”*

2003 report from the National Research Council

Gerald Fink, Chair

- *“Globalization, Biosecurity and the Future of the Life Sciences”*

2005 report from the Institute of Medicine and NRC

Stanley M. Lemon and David A. Relman, Co-chairs



The 2003 “Fink Report”



“Biotechnology Research in an Age of Terrorism”
2003 report from the National Research Council
Gerald Fink, Chair

- **Recommendation 1: Educating the Scientific Community**
 - Awareness of the dual use phenomenon is not widespread in the biological sciences.
- **Recommendation 2: Review of Plans for Experiments**
 - Identified 7 types of “experiments of concern”
- **Recommendation 3: Review at the Publication Stage**
- **Recommendation 4: Creation of a *National Science Advisory Board for Biosecurity* (NSABB)**
 - NSABB was established in late 2004 by then-Secretary Leavitt (HHS)

The “7 Deadly Sins” from 2003 Fink Report

Recommendation 2: Review of Plans for Experiments

- **“We recommend that the Department of Health and Human Services (DHHS) augment the already established system for review of experiments involving recombinant DNA conducted by the National Institutes of Health to create a review system for seven classes of experiments (The Experiments of Concern) involving microbial agents that raise concerns about their potential for misuse.”**

“Experiments of Concern” would:

- demonstrate how to render a vaccine ineffective
- confer resistance to therapeutically useful antibiotics or anti-viral agents
- enhance the virulence of a pathogen or render a nonpathogen virulent
- increase transmissibility of a pathogen
- alter the host range of a pathogen
- enable the evasion of diagnostic or detection modalities
- enable the weaponization of a biological agent or toxin





NATIONAL STRATEGY
for
COUNTERING
BIOLOGICAL THREATS

National Security Council

NOVEMBER 2009



www.whitehouse.gov/sites/default/files/National_Strategy_for_Countering_BioThreats.pdf



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National Science Advisory Board for Biosecurity

- **NSABB is a federal advisory committee, established in 2004 to:**
 - Recommend strategies for oversight of federally conducted or supported dual use research
 - Raise awareness of dual use issues
- **NSABB advises heads of Federal entities that have a role or interest in life sciences research**
- **25 non-governmental voting members with broad expertise**
- ***Ex officio* members from Federal departments and agencies**

Why “Gain of Function Experiments”?

Transmissibility

- **Essential for pathogen success**
- **Possible relation to virulence**
- **Genetics and evolution of transmissibility poorly understood**
- **Are emerging pathogens with broad host range more likely to become transmissible human-to-human ?**



THE FOURTH ESWI
INFLUENZA
CONFERENCE

11-14 SEPTEMBER 2011 | MALTA

THE INFLUENZA TIMES

According to estimates, this special because young scientists new data and all the scientific sessions will be

mechanism for

in Melbourne, Tennessee and the University of Melbourne in Australia delivered the keynote address. Doherty gave a wide-ranging talk that presented influenza research performed over the past several decades. He summarized research findings, added his own conclusions and raised questions requiring further scientific investigation.

According to Doherty, the real problem with the influenza virus is that it's highly contagious but the infected don't necessarily feel sick while they're infectious. On the plus side, unlike HIV, influenza infections are "self-limiting" provided one can limit the damage. "We just need to get people through the acute phase," said Doherty. For this purpose, Doherty ended his keynote with a challenge: "Can we make a universal vaccine?" ■



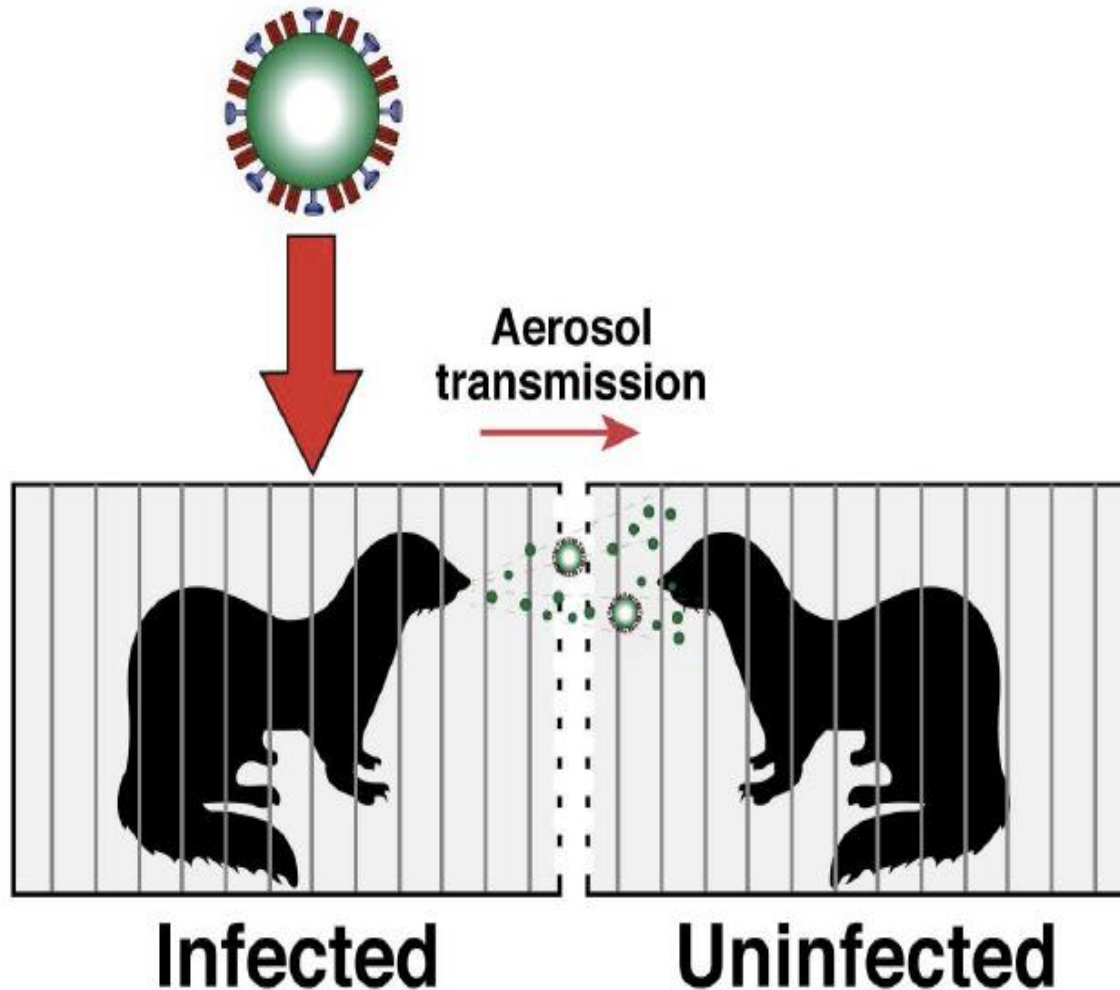
Ron Fouchier



A “stupid” experiment leads to a valuable result. Fouchier and his team’s biggest discovery, however, was based on what he termed a “stupid” experiment. He and his team introduced mutations, under strict laboratory safety procedures, by reverse genetics into laboratory ferrets. They then collected a nasal wash from each infected ferret and inoculated another ferret after a few days. They repeated this process ten times. The result? H5N1 had been transmitted to three out of four ferrets. “This virus is airborne and as efficiently transmitted as the seasonal virus,” said Fouchier. His research team found that only 5 mutations, 3 by reverse genetics and 2 by repeated transmission, were enough to produce this result. “This is very bad news, indeed,” said Fouchier. ■

H5N1 Influenza Transmissibility Studies: Fouchier and Kawaoka

Modified H5N1



Yoshi Kawaoka and Ron Fouchier



USG Definition of DURC

Research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security.

Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets

Sander Herfst,¹ Eefje J. A. Schrauwen,² Martin Linster,² Salin Chutinimitkul,² Emmie de Wit,^{2*} Vincent J. Munster,^{2*} Erin M. Sorrell,² Theo M. Bestebroer,² David F. Burke,² Derek J. Smith,^{1,2,3} Gaus F. Rimmelzwaan,² Albert D. M. E. Osterhaus,² Ron A. M. Fouchier^{1†}

Highly pathogenic avian influenza A/H5N1 virus can cause morbidity and mortality in humans but thus far has not acquired the ability to be transmitted by aerosol or respiratory droplet ("airborne transmission") between humans. To address the concern that the virus could acquire this ability under natural conditions, we genetically modified A/H5N1 virus by site-directed mutagenesis and subsequent serial passage in ferrets. The genetically modified A/H5N1 virus acquired mutations during passage in ferrets, ultimately becoming airborne transmissible in ferrets. None of the recipient ferrets died after airborne infection with the mutant A/H5N1 viruses. Four amino acid substitutions in the host receptor-binding protein hemagglutinin, and one in the polymerase complex protein basic polymerase 2, were consistently present in airborne-transmitted viruses. The transmissible viruses were sensitive to the antiviral drug oseltamivir and reacted well with antisera raised against H5 influenza vaccine strains. Thus, avian A/H5N1 influenza viruses can acquire the capacity for airborne transmission between mammals without recombination in an intermediate host and therefore constitute a risk for human pandemic influenza.

Influenza A viruses have been isolated from many host species, including humans, pigs, horses, dogs, marine mammals, and a wide range of domestic birds, yet wild birds in the orders Anseriformes (ducks, geese, and swans) and Charad-

riiformes (gulls, terns, and waders) are thought to form the virus reservoir in nature (*1*). Influenza A viruses belong to the family Orthomyxoviridae; these viruses have an RNA genome consisting of eight gene segments (*2, 3*). Segments 1 to 3 encode the polymerase proteins: basic polymerase 2 (PB2), basic polymerase 1 (PB1), and acidic polymerase (PA), respectively. These proteins form the RNA-dependent RNA polymerase complex responsible for transcription and replication of the viral genome. Segment 2 also encodes a

second small protein, PB1-F2, which has been implicated in the induction of cell death (*4, 5*). Segments 4 and 6 encode the viral surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), respectively. HA is responsible for binding to sialic acids (SAs), the viral receptors on host cells, and for fusion of the viral and host cell membranes upon endocytosis. NA is a sialidase, responsible for cleaving SAs from host cells and virus particles. Segment 5 codes for the nucleocapsid protein (NP) that binds to viral RNA and, together with the polymerase proteins, forms the ribonucleoprotein complexes (RNPs). Segment 7 codes for the viral matrix structural protein M1 and the ion-channel protein M2 that is incorporated in the viral membrane. Segment 8 encodes the nonstructural protein NS1 and the nucleic export protein (NEP) previously known as NS2. NS1 is an antagonist of host innate immune responses and interferes with host gene expression, whereas NEP is involved in the nuclear export of RNPs into the cytoplasm before virus assembly (*2, 3*).

Influenza A viruses show pronounced genetic variation of the surface glycoproteins HA and NA (*1*). Consequently, the viruses are classified based on the antigenic variation of the HA and NA proteins. To date, 16 major antigenic variants of HA and 9 of NA have been recognized in wild birds and are found in numerous combinations designated as virus subtypes (for instance, H1N1, H5N1, H7N7, and H16N3), which are used in influenza A virus classification and nomenclature (*1, 6*). This classification system is biologically relevant, as natural host antibodies that recognize

¹Department of Virology, Erasmus Medical Center, Rotterdam, The Netherlands. ²Department of Zoology, University of Cambridge, Cambridge, UK. ³Fogarty International Center, National Institutes of Health (NIH), Bethesda, MD 20892, USA.

†To whom correspondence should be addressed. E-mail: r.fouchier@erasmusmc.nl

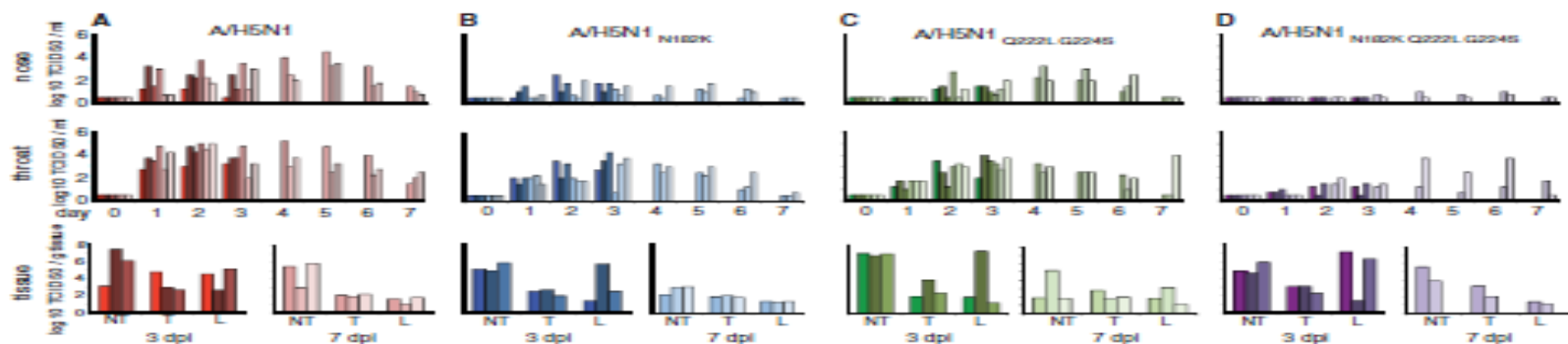


Fig. 1. In experiment 1, we inoculated groups of six ferrets intranasally with 1×10^5 TCID₅₀ of (A) influenza A/H5N1_{wt/dp1} virus and the three mutants (B) A/H5N1_{HA N182K}, (C) A/H5N1_{HA Q222L, Q224S}, and (D) A/H5N1_{HA N182K, Q222L, Q224S}. Three animals were euthanized at day 3 for tissue sampling and at day 7, when this experiment was stopped. Virus titers were measured daily in nose swabs (top) and throat swabs (middle) and also on 3 and 7 dpi in respiratory tract tissues (bottom) of individual ferrets. Virus titers in swabs and nasal turbinates (NT), trachea (T), and lungs (L) were determined by end-point titration in MDCK cells. [One animal inoculated with A/H5N1_{HA N182K, Q222L, Q224S} died at 1 dpi due to circumstances not related to the experiment (D).] (Top two rows) Virus

shedding from the URT as determined by virus titers in nasal and throat swabs was highest in A/H5N1_{wt/dp1}-inoculated animals. The mutant that yielded the highest virus titers during the 7-day period was A/H5N1_{HA Q222L, Q224S}, but titers were -1 log lower than for the A/H5N1_{wt/dp1}-inoculated animals. In the first 3 days, when six animals per group were present, no significant differences were observed between A/H5N1_{HA N182K} and A/H5N1_{HA Q222L, Q224S}-inoculated animals, as calculated by comparing the viral titer (Mann-Whitney test, $P = 0.589$ and 0.818 for nose and throat titers, respectively). (Bottom row) No marked differences in virus titers in respiratory tissues were observed between the four groups. Each bar color denotes a single animal.

Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets

Masaki Imai¹, Tokiko Watanabe^{1,2}, Masato Hatta¹, Subash C. Das¹, Makoto Ozawa^{1,3}, Kyoko Shinya⁴, Gongxun Zhong¹, Anthony Hanson¹, Hiroaki Katsuma⁵, Shinji Watanabe^{1,2}, Chengjun Li¹, Eiryo Kawakami², Shinya Yamada², Maki Kiso², Yasuo Suzuki⁶, Eileen A. Maher¹, Gabriele Neumann¹ & Yoshihiro Kawaoka^{1,2,3,5}

Highly pathogenic avian H5N1 influenza A viruses occasionally infect humans, but currently do not transmit efficiently among humans. The viral haemagglutinin (HA) protein is a known host-range determinant as it mediates virus binding to host-specific cellular receptors^{1–3}. Here we assess the molecular changes in HA that would allow a virus possessing subtype H5 HA to be transmissible among mammals. We identified a reassortant H5 HA/H1N1 virus—comprising H5 HA (from an H5N1 virus) with four mutations and the remaining seven gene segments from a 2009 pandemic H1N1 virus—that was capable of droplet transmission in a ferret model. The transmissible H5 reassortant virus preferentially recognized human-type receptors, replicated efficiently in ferrets, caused lung lesions and weight loss, but was not highly pathogenic and did not cause mortality. These results indicate that H5 HA can convert to an HA that supports efficient viral transmission in mammals; however, we do not know whether the four mutations in the H5 HA identified here would render a wholly avian H5N1 virus transmissible. The genetic origin of the remaining seven viral gene segments may also critically contribute to transmissibility in mammals. Nevertheless, as H5N1 viruses continue to evolve and infect humans, receptor-binding variants of H5N1 viruses with pandemic potential, including avian–human reassortant viruses as tested here, may emerge. Our findings emphasize the need to prepare for potential pandemics caused by influenza viruses possessing H5 HA, and will help individuals conducting surveillance in regions with circulating H5N1 viruses to recognize key residues that predict the pandemic potential of isolates, which will inform the development, production and distribution of effective countermeasures.

Although H5N1 viruses continue to cause outbreaks in poultry and there are cases of human infection in Indonesia, Vietnam, Egypt and elsewhere (http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/index.html), they have not acquired the ability to cause human-to-human transmission. Investment in H5N1 vaccines has therefore been questioned. However, because humans lack immunity to influenza viruses possessing an H5 HA, the emergence of a transmissible H5-HA-possessing virus would probably cause a pandemic. To prepare better for such a scenario, it is critical that we understand the molecular changes that may render H5-HA-possessing viruses transmissible in mammals. Such knowledge would allow us to monitor circulating or newly emerging variants for their pandemic potential, focus eradication efforts on viruses that already have acquired subsets of molecular changes critical for transmission in mammals, stockpile antiviral compounds in regions where such viruses circulate, and initiate vaccine generation and large-scale production

before a pandemic. Therefore, we studied the molecular features that would render H5-HA-possessing viruses transmissible in mammals.

Previous studies suggested that HA has a major role in host-range restriction of influenza A viruses^{1–3}. The HA of human isolates preferentially recognizes sialic acid linked to galactose by $\alpha 2,6$ -linkages (Sia $\alpha 2,6$ Gal), whereas the HA of avian isolates preferentially recognizes sialic acid linked to galactose by $\alpha 2,3$ -linkages (Sia $\alpha 2,3$ Gal)³. A small number of avian H5N1 viruses isolated from humans show limited binding to human-type receptors, a property conferred by several amino acid changes in HA^{4–9}. None of the H5N1 viruses tested transmitted efficiently in a ferret model^{10–12}, although, while our paper was under review, one study¹⁴ reported that a virus with a mutant H5 HA and a neuraminidase (NA) of a human virus in the H5N1 virus background caused respiratory droplet transmission in one of two contact ferrets.

To identify novel mutations in avian H5 HAs that confer human-type receptor-binding preference, we introduced random mutations into the globular head (amino acids 120–259 (H3 numbering), which includes the receptor-binding pocket) of A/Vietnam/1203/2004 (H5N1; VN1203) HA (Supplementary Fig. 1). Although this virus was isolated from a human, its HA retains avian-type receptor-binding properties^{6,15}. We also replaced the multibasic HA cleavage sequence with a non-virulent-type cleavage sequence, allowing us to perform studies in biosafety level 2 containment (http://www.who.int/csr/resources/publications/influenza/influenzaRMD2003_5.pdf). The mutated polymerase chain reaction (PCR) products were cloned into RNA polymerase I plasmids¹⁶ containing the VN1203 HA complementary DNA, which resulted in *Escherichia coli* libraries representing the randomly generated HA variants. Sequence analysis of 48 randomly selected clones indicated an average of 1.0 amino acid changes per HA globular head (data not shown). To generate an H5N1 virus library, plasmids for the synthesis of the mutated HA gene and the unmodified NA gene of VN1203 were transfected into human embryonic kidney (293T) cells together with plasmids for the synthesis of the six remaining viral genes of A/Puerto Rico/8/34 (H1N1; PR8), a laboratory-adapted human influenza A virus.

Turkey red blood cells (TRBCs; which possess both Sia $\alpha 2,6$ Gal and Sia $\alpha 2,3$ Gal on their surface (data not shown)) were treated with *Salmonella enterica* serovar Typhimurium LT2 sialidase, which preferentially removes $\alpha 2,3$ -linked sialic acid (that is, avian-type receptors), creating TRBCs that predominantly possess Sia $\alpha 2,6$ Gal on the cell surface (Sia $\alpha 2,6$ -TRBCs; Supplementary Fig. 2). The virus library was then adsorbed to Sia $\alpha 2,6$ -TRBCs at 4 °C and extensively washed to remove non-specifically or weakly bound viruses. Bound viruses were eluted by incubation at 37 °C for 30 min, and then diluted to approximately ~0.5 viruses per well (on the basis of a pilot experiment that

¹Department of Pathobiological Sciences, University of Wisconsin–Madison, Madison, Wisconsin 53711, USA. ²ERATO Infectious-Induced Host Responses Project, Saitama 332-0012, Japan. ³Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo 108-8539, Japan. ⁴Department of Microbiology and Infectious Diseases, Kobe University, Hyogo 650-0017, Japan. ⁵Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8539, Japan. ⁶Health Science HHS, College of Life and Health Sciences, Chubu University, Kasugai, Aichi 487-8501, Japan.



Airborne Transmission of Highly Pathogenic H7N1 Influenza Virus in Ferrets

Troy C. Sutton,^a Courtney Finch,^a Hongxia Shao,^{**} Matthew Angel,^a Hongjun Chen,^a Ilaria Capua,^b Giovanni Cattoli,^b Isabella Monne,^b Daniel R. Perez^a

Department of Veterinary Medicine, University of Maryland, College Park, and Virginia-Maryland College of Veterinary Medicine, Maryland Campus, College Park, Maryland, USA^a; Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy^b

ABSTRACT

Avian H7 influenza viruses are recognized as potential pandemic viruses, as personnel often become infected during poultry outbreaks. H7 infections in humans typically cause mild conjunctivitis; however, the H7N9 outbreak in the spring of 2013 has resulted in severe respiratory disease. To date, no H7 viruses have acquired the ability for sustained transmission among humans. Airborne transmission is considered a requirement for the emergence of pandemic influenza, and advanced knowledge of the molecular changes or signature required for transmission would allow early identification of pandemic vaccine seed stocks, screening and stockpiling of antiviral compounds, and eradication efforts focused on flocks harboring threatening viruses. Thus, we sought to determine if a highly pathogenic influenza A H7N1 (A/H7N1) virus with no history of human infection could become capable of airborne transmission among ferrets. We show that after 10 serial passages, A/H7N1 developed the ability to be transmitted to cohoused and airborne contact ferrets. Four amino acid mutations (PB2 T81I, NP V284M, and M1 R95K and Q211K) in the internal genes and a minimal amino acid mutation (K/R313R) in the stalk region of the hemagglutinin protein were associated with airborne transmission. Furthermore, transmission was not associated with loss of virulence. These findings highlight the importance of the internal genes in host adaptation and suggest that natural isolates carrying these mutations be further evaluated. Our results demonstrate that a highly pathogenic avian H7 virus can become capable of airborne transmission in a mammalian host, and they support ongoing surveillance and pandemic H7 vaccine development.

Pathogenicity and transmissibility of reassortant H9 influenza viruses with genes from pandemic H1N1 virus

Chuanling Qiao,^{1,2†} Qinfang Liu,^{1†} Bhupinder Bawa,¹ Huigang Shen,¹ Wenbao Qi,^{1‡} Ying Chen,¹ Chris Ka Pun Mok,³ Adolfo García-Sastre,^{4,5,6} Jürgen A. Richt¹ and Wenjun Ma¹

¹Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS 66506, USA

²Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, PR China

³Centre of Influenza Research, School of Public Health, The University of Hong Kong, Hong Kong SAR

⁴Department of Microbiology, Mount Sinai School of Medicine, NY 10029, USA

⁵Department of Medicine, Division of Infectious Diseases, Mount Sinai School of Medicine, NY 10029, USA

⁶Global Health and Emerging Pathogens Institute, Mount Sinai School of Medicine, NY 10029, USA

Both H9N2 avian influenza and 2009 pandemic H1N1 viruses (pH1N1) are able to infect humans and swine, which has raised concerns that novel reassortant H9 viruses with pH1N1 genes might be generated in these hosts by reassortment. Although previous studies have demonstrated that reassortant H9 viruses with pH1N1 genes show increased virulence in mice and transmissibility in ferrets, the virulence and transmissibility of reassortant H9 viruses in natural hosts such as chickens and swine remain unknown. This study generated two reassortant H9 viruses (H9N2/CA09 and H9N1/CA09) in the background of the pH1N1 A/California/04/2009 (CA09) virus by replacing either both the haemagglutinin (HA) and neuraminidase (NA) genes or only the HA gene with the respective genes from the A/quail/Hong Kong/G1/1997 (H9N2) virus and evaluated their replication, pathogenicity and transmission in chickens and pigs compared with the parental viruses. Chickens that were infected with the parental H9N2 and reassortant H9 viruses seroconverted. The parental H9N2 and reassortant H9N2/CA09 viruses were transmitted to sentinel chickens, but H9N1/CA09 virus was not. The parental H9N2 replicated poorly and was not transmitted in pigs, whereas both H9N2/CA09 and H9N1/CA09 viruses replicated and were transmitted efficiently in pigs, similar to the pH1N1 virus. These results demonstrated that reassortant H9 viruses with pH1N1 genes show enhanced replication and transmissibility in pigs compared with the parental H9N2 virus, indicating that they may pose a threat for humans if such reassortants arise in swine.

Correspondence

Wenjun Ma

wma@vet.k-state.edu

Juergen Richt

jricht@vet.k-state.edu

H5N1 Hybrid Viruses Bearing 2009/H1N1 Virus Genes Transmit in Guinea Pigs by Respiratory Droplet

Ying Zhang,^{1*} Qianyi Zhang,^{1,2*} Huihui Kong,¹ Yongping Jiang,¹ Yuwei Gao,¹ Guohua Deng,¹ Jianzhong Shi,¹ Guobin Tian,¹ Liling Liu,¹ Jinxiong Liu,¹ Yuntao Guan,¹ Zhigao Bu,¹ Hualan Chen^{1,2,†}

In the past, avian influenza viruses have crossed species barriers to trigger human pandemics by reassorting with mammal-infective viruses in intermediate livestock hosts. H5N1 viruses are able to infect pigs, and some of them have affinity for the mammalian type α -2,6-linked sialic acid airway receptor. Using reverse genetics, we systematically created 127 reassortant viruses between a duck isolate of H5N1, specifically retaining its hemagglutinin (*HA*) gene throughout, and a highly transmissible, human-infective H1N1 virus. We tested the virulence of the reassortants in mice as a correlate for virulence in humans and tested transmissibility in guinea pigs, which have both avian and mammalian types of airway receptor. Transmission studies showed that the H1N1 virus genes encoding acidic polymerase and nonstructural protein made the H5N1 virus transmissible by respiratory droplet between guinea pigs without killing them. Further experiments implicated other H1N1 genes in the enhancement of mammal-to-mammal transmission, including those that encode nucleoprotein, neuraminidase, and matrix, as well as mutations in H5 *HA* that improve affinity for humanlike airway receptors. Hence, avian H5N1 subtype viruses do have the potential to acquire mammalian transmissibility by reassortment in current agricultural scenarios.

Avian influenza viruses continue to evolve and spread, perpetuating the fear of an influenza pandemic if they acquire the ability to transmit efficiently among humans. The influenza virus genome comprises eight gene segments: basic polymerase 2 (*PB2*), basic polymerase 1 (*PB1*), acidic polymerase (*PA*), hemagglutinin (*HA*), nucleoprotein (*NP*), neuraminidase (*NA*), matrix (*M*), and nonstructural protein (*NS*). Hemagglutinin and neuraminidase are integral membrane proteins. The *HA* of human-infective

influenza subtypes preferentially recognizes α -2,6-linked sialic acids (SAs) (humanlike receptor), whereas the *HA* of avian-infective influenza subtypes preferentially recognizes α -2,3-linked SAs (avian-like receptor) (*1*). Combinations of amino acid changes such as 158D/224K/226L, 196R/226L/228S, or 110Y/160A/226L/228S (H3 numbering used throughout; see fig. S6) in *HA* protein can allow H5N1 viruses to recognize α -2,6-linked SAs, thereby conferring viral transmission between ferrets (*2–4*).

When two different influenza viruses infect the same cell, their genes can reassort to produce new viral strains. Historically, such reassortment has led to the emergence and spread of pandemic viruses in immunologically naïve human populations (*5–8*). A previous study with an H5N1 virus and a human H3N2 virus suggested that reassortments between these two subtypes to produce a

dangerous virus would be rare (*9*). However, both avian H5N1 and human 2009/H1N1 viruses have been found in pigs (*10–14*), so we asked: Could an H5N1 reassortant between avian H5N1 and the highly transmissible 2009/H1N1 virus become transmissible among mammals and potentially cause a human pandemic?

H5N1 influenza viruses were handled in the enhanced animal biosafety laboratory level 3 (ABSL3+) facility at the Harbin Veterinary Research Institute, China (*15*). All experimental studies with live H5N1 viruses were performed before the moratorium on such studies was in place (*16, 17*). Details of the biosafety and biosecurity measures taken and the dates on which the experiments were performed are provided in the supplementary materials.

We used two influenza viruses isolated in China: the H5N1 virus A/duck/Guangxi/35/2001 [DK/35(H5N1)] and the H1N1 virus A/Sichuan/1/2009 [SC/09(H1N1)]. DK/35(H5N1) is highly pathogenic for both chickens and mice (*18*). It transmits by direct contact among guinea pigs when they are housed together (*19*) but does not transmit between guinea pigs by respiratory droplet (Fig. 1A). We previously identified two molecular changes that are critical for the contact transmission of DK/35(H5N1) among guinea pigs: the asparagine residue at position 701 (701N) in *PB2* and the alanine residue at position 160 (160A) in *HA* (*19*). The mutation of 160A, resulting in the absence of glycosylation at positions 158 to 160 in *HA*, permits virus binding to α -2,6-linked SAs (*19, 20*). Receptor specificity testing, using a solid-phase binding assay with four different glycans, indicated that DK/35(H5N1) binds to both α -2,3-linked SAs and α -2,6-linked SAs, and that its affinity to α -2,3-linked SAs is higher than to α -2,6-linked SAs (fig. S1A). SC/09(H1N1) was the first virus isolated in China during the 2009 influenza pandemic and transmits efficiently among guinea pigs by respiratory droplet (Fig. 1B) (*21*).

Using plasmid-based reverse genetics (*22–24*), we generated all possible reassortants possessing the H5 *HA* gene [i.e., 127 hybrid viruses between DK/35(H5N1) and SC/09(H1N1), ²⁷ minus one

¹State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, People's Republic of China. ²College of Veterinary Medicine, Gansu Agricultural University, Lanzhou 730030, People's Republic of China.

*These authors contributed equally to this work.

†Corresponding author. E-mail: chenhualan@caas.cn

Some Laboratory Incidents

British smallpox cases, 1972, 1978	Associated with smallpox laboratory at University of Birmingham
The “re-emergence” of H1N1 human influenza (1977)	Error in vaccine or challenge virus in China?
Venezuelan Equine Encephalitis (1995)?	Outbreak in Venezuela, 1995; source of virus unclear
SARS	Singapore (1), Taiwan (1), Beijing (4)
H2N2 (1957 pandemic influenza), CAP lab competency testing samples (2005)	
Foot and Mouth Disease (FMD) from Pirbright, UK (2007)	

M. Furmanski. “Escaped Viruses”: Laboratory Escapes and “Self-fulfilling prophecy” Epidemics. Working Paper (Feb. 2014), Center for Arms Control and Nonproliferation, armscontrolcenter.org/Escaped_Viruses-final_2-17-14.pdf, and CDC

Laboratory Acquired Infections with Select Agents, US, 2004-2010

Table 4

Laboratory Acquired Infections caused by BSATs between 2004-2010.

The annual distribution, type of select agent, type of entity and type of containment facility in which the 11 laboratory acquired infections occurred that were reported to CDC between 2004 -2010.

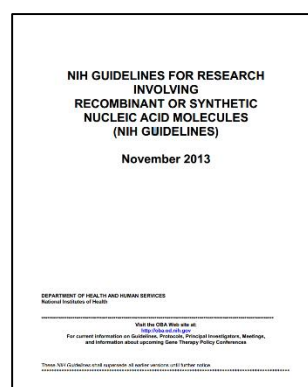
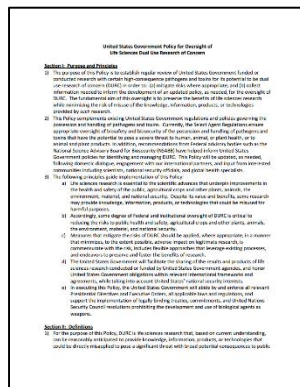
Year	Agent	# Cases	Entity type	Laboratory Type
2004	<i>Brucella melitensis</i>	1	Registered	BSL 2
2004	<i>Coccidioides</i> species	1	Registered	BSL 3
2004	<i>Francisella tularensis</i>	3	Registered	BSL 2
2007	<i>Brucella melitensis</i>	1	Registered	BSL 3
2007	<i>Brucella melitensis</i>	1	Exempt	BSL 2
2008	<i>Brucella melitensis</i>	1	Registered	BSL 3
2009	<i>Francisella tularensis</i>	1	Registered	BSL 3
2010	<i>Brucella suis</i>	1	Exempt	BSL 2
2010	<i>Brucella suis</i>	1	Exempt	BSL 2

It all started with Asilomar ...



U.S. Frameworks that Address GOF

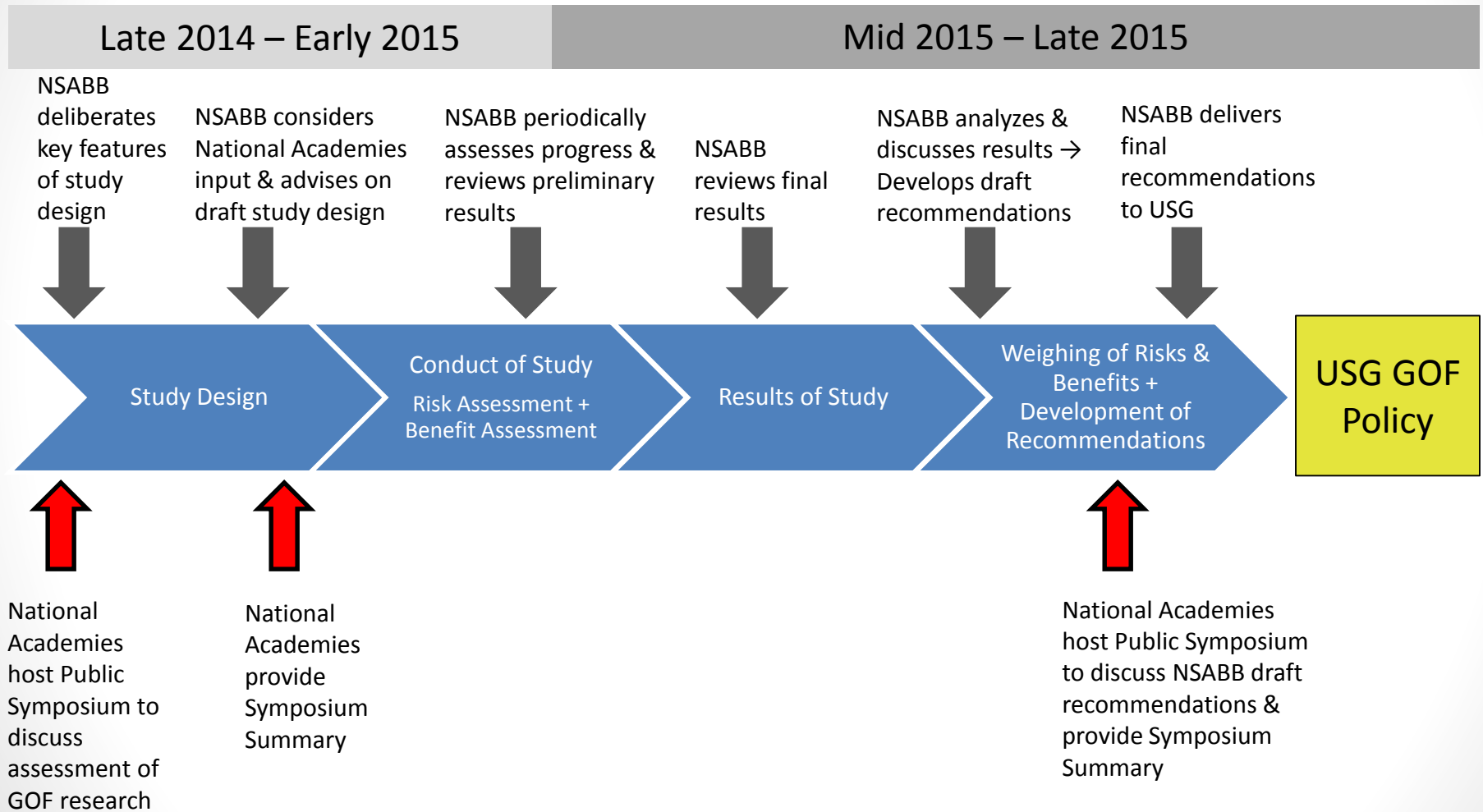
- *HHS Framework for Highly Pathogenic Avian Influenza Research (2012)*
- *USG Policy for Oversight of Life Sciences Dual Use Research of Concern (March 29, 2012)*
- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (November 2013)*
- *USG Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (September 24, 2014)*



For More Information

- Holdren/Monaco memorandum on *Enhancing Biosafety and Biosecurity in the United States* (August 2014)
http://www.whitehouse.gov/sites/default/files/microsites/ostp/enhancing_biosafety_and_biosecurity_19aug2014_final.pdf
- *U.S. Government Deliberative Process and Funding Pause on Certain Types of Gain-of-Function Research* (October 2014)
<http://www.phe.gov/s3/dualuse/Pages/default.aspx>
- *USG Policy for Oversight of Life Sciences Dual Use Research of Concern* (March 2012)
<http://www.phe.gov/s3/dualuse/Documents/us-policy-durc-032812.pdf>
- *USG Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern* (September 2014)
<http://www.phe.gov/s3/dualuse/Documents/durc-policy.pdf>

Estimated Timeline*



*The USG intends for these efforts to occur as expeditiously as possible, and dates are subjects to change based on the deliberative process.

Website Resources

**CDC Journal “Emerging Infectious Diseases”:
www.cdc.gov/eid/**

**WHO outbreak information:
www.who.int/csr/don/en/**

**ProMED-mail:
www.promedmail.org/**

**CIDRAP
(Center for Infectious Disease Research and
Policy, University of Minnesota):
<http://www.cidrap.umn.edu/>**

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Grandma's Only Movie Watched Again

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FDA APPROVES SALMONELLA

Pathogen 'Now Part Of A Well-Balanced Diet'

WASHINGTON—Calling it “perfectly safe for the most part,” and “not nearly as destructive or fatal as you’d think,” the Food and Drug Administration approved the enterobacteria salmonella for human consumption this week.

The federal agency, which has struggled in recent years to contain the food-borne pathogen, and repeatedly failed to prevent tainted products from reaching store shelves, announced Monday that salmonella was now com-

pletely okay for all Americans to enjoy.

“Rigorous testing has shown that salmonella is...fine,” FDA director of food safety Stephen Sundlof said. “In fact, our research indicates that there’s

see FDA, page 8



FDA director Stephen Sundlof plays the bacteria for eating, drinking, and applying directly to the skin.

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Outbreaks of E. coli O104:H4 infection: update 30

22-07-2011

The number of new cases of *Escherichia coli* O104:H4 infection in Germany and France is much diminished. The figures reflect some delayed reporting, and the evidence indicates that the outbreak, which took 50 lives in Germany alone, is nearly over.

WHO/Europe will continue to monitor developments with its partner organizations and, when appropriate, report on this web site on the progress of investigations into the source of the infection.

In total, the table shows that 16 countries in Europe and North America had reported 4075 cases and 50 deaths as of 21 July at 18:00 CET.

Country	HUS		EHEC	
	Cases	Deaths	Cases	Deaths
Austria	1	0	4	0
Canada	0	0	1	0
Czech Republic	0	0	1	0
Denmark	10	0	15	0
France	7	0	10	0
Germany	857	32	3078*	16
Greece	0	0	1	0
Luxembourg	1	0	1	0
Netherlands	4	0	7	0
Norway	0	0	1	0
Poland	2	0	1	0
Spain	1	0	1	0
Sweden	18	1	35	0
Switzerland	0	0	5	0
United Kingdom	3	0	4	0
United States of America	4	1	2	0
Total	908	34	3167	16

See also

Outbreaks of E. coli O104:H4 infection
Previous updates and background information

External resources

European Food Safety Authority
Press release of 5 July 2011

Outbreaks of E. coli O104:H4 infection

► Latest update

World's largest E coli outbreak kills 14 in Germany

More than 300 seriously ill in Germany as E coli bacterium spreads to other northern European countries

Reuters

guardian.co.uk, Monday 30 May 2011 18.36 BST

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The Emerging Infections Two-Step

Opportunities increasing for both steps:

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- Rural to urban migration**
- Internal displacement**
- Globalization of people and goods, travel, international migration**