MediLabSecure Global Meeting

Commemorating 10 Years of Dedicated Efforts & Achievements in Vector-Borne Diseases Preparedness

June 11-13th, 2024 — Paris, France

Dual-use Research

Funded by the European Union

What is « dual use » research?

- What is "Dual use research"?
- What are gain of function (GOF)?
- What are the risks and possible consequences?
- How to prevent them?
- Examples?

Biosafety & Biosecurity

Biosafety:

Principles, technologies, and practices implemented to prevent inadvertent exposure, or release, of biological agents (WHO).

All measures and practices to protect people and the environment from the consequences of infection, poisoning, or the spread of microorganisms or toxins.

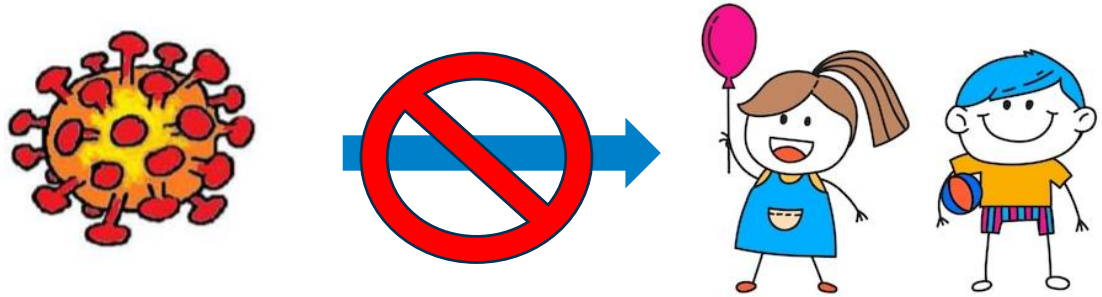
Biosecurity:

Principles, technologies, and practices implemented for the protection, control, and responsibility of biological material and/or the equipment, skills, and data related to their handling.

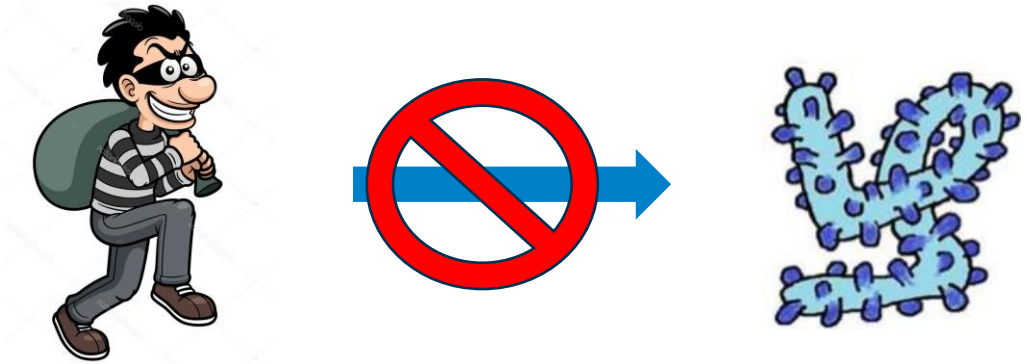
Biosecurity aims to prevent unauthorized access, loss, theft, misuse, diversion, or dissemination (WHO). Diversion or misuse of all or part of micro-organisms or toxins with the aim of causing illness or death of human beings (Article R.5139-19 of the French Public Health Code).

Biosafety & Biosecurity

Biosafety: To keep bad bugs from People!



Biosecurity : To keep bad people from bugs!



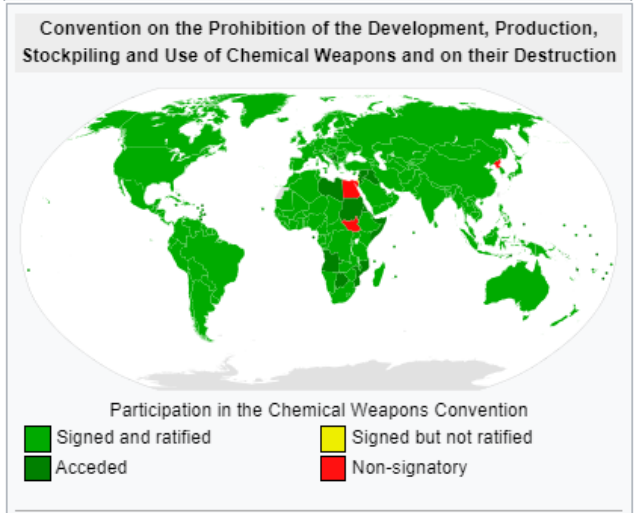
Why Biosecurity?

1. Respect of international conventions and agreement.

2. Countering the proliferation of biological weapons.

3. Prevent the risks or threats of "bioterrorism".

4. To preserve our country's fundamental economic interests and defense capabilities.



➢ **These obligations derive from**:

❖ **The Biological Weapons Convention** (BWC): 183/197 States have ratified or acceded to the Convention.

❖ **Chemical Weapons Convention** (CWC)

https://en.wikipedia.org/wiki/Chemical_Weapons_Convention
https://en.wikipedia.org/wiki/Biological_Weapons_Convention

Why is Biosecurity necessary?

Bioweapons

- Biopreparat
- Project coast
- Unit 731
- etc.

Bioterror

1984 Rajneeshee – The Dalles

Aum Shinrikyō

Propaganda

How to implement Biosecurity?

❖ **Regulation and good laboratory practices:**

Confinement, access restriction, traceability….

❖ **Control of exchanges for Dual-use goods and technologies:**

➢ Physical assets (pathogens, laboratory equipment, …)

➢ Intangible goods (knowledge, know-how, methods and processes….).

❖ **Remark**: following national and international regulation!

Dual Use Research

- **Research that can benefit both the civil and the military sectors.**

-Biology and health

-Aerospatiale

-Information and communication

-Etc.

Dual Use Research of Concern (DURC)

"Research that [is intended to provide a clear benefit, but] based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment, or materiel or national security."

*NSABB 2007 (***National Science Advisory Board for Biosecurity (NSABB))**

Research whose "misuse" or "accidental leak" could pose an unacceptable threat.

- unacceptable in terms of **consequences** for Public Health or the environment
- **ethically unacceptable**, and therefore **scientifically unjustifiable**

http://www.bu.edu/orc/ini.a.ves/durc/, http://osp.od.nih.gov/officebiotechnology-ac.vi.es/biosecurity/dual-use-research-concern , http://www.phe.gov/s3/dualuse/Documents/durc-companion-guide.pdf (A Companion Guide to the United States Government Policies for Oversight of Life Sciences Dual Use Research of Concern Prepared by the National Institutes of Health on behalf of the United States Government, SEPTEMBER 2014)

Few example

2005: Recreating the 1918 Spanish Flu H1N1

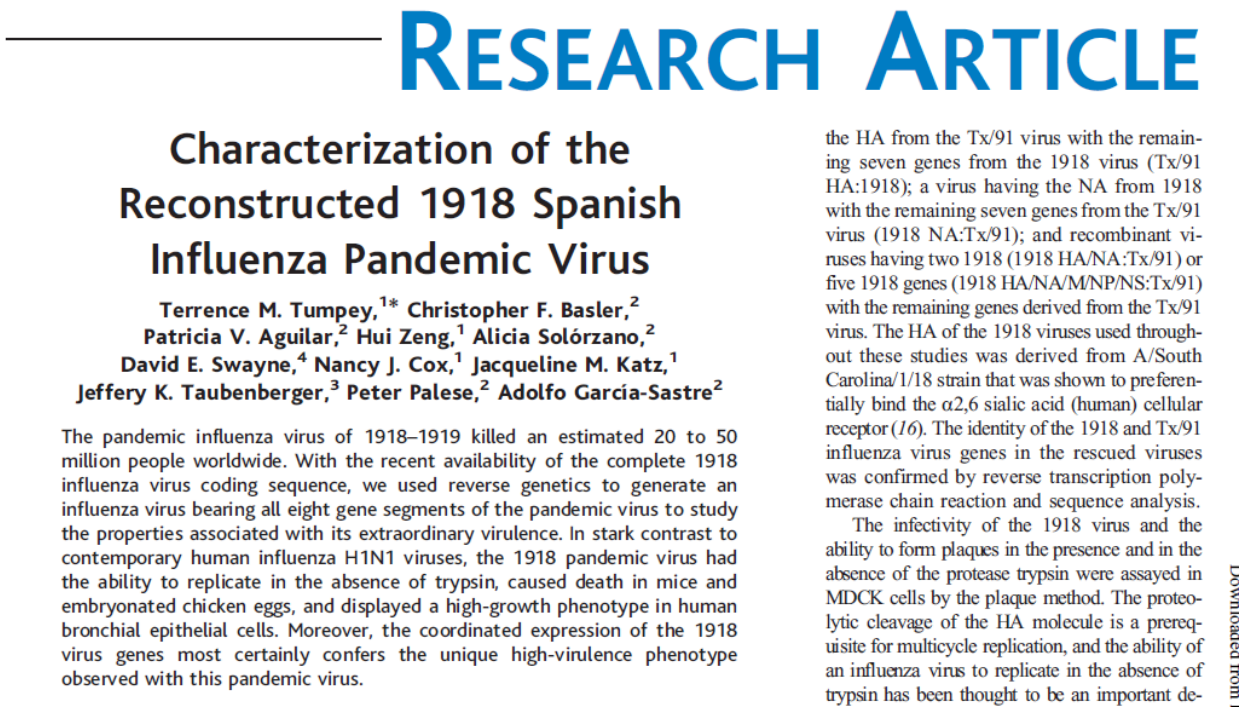
"we used reverse genetics to generate an influenza virus bearing all eight gene segments of the pandemic virus to study the properties associated with its extraordinary virulence."

Scientific justification:

- To understand why and how the H1N1 virus was so deadly.
- To be better prepared to prevent a future pandemic.

Possible misuse:

- Availability of a deadly pathogen
- Blueprint to create a deadly pathogen



RESEARCH ARTICLE

Characterization of the Reconstructed 1918 Spanish Influenza Pandemic Virus

Terrence M. Tumpey,[1]* Christopher F. Basler,[2] Patricia V. Aguilar,[2] Hui Zeng,[1] Alicia Solórzano,[2] David E. Swayne,[4] Nancy J. Cox,[1] Jacqueline M. Katz,[1] Jeffery K. Taubenberger,[3] Peter Palese,[2] Adolfo García-Sastre[2]

The pandemic influenza virus of 1918–1919 killed an estimated 20 to 50 million people worldwide. With the recent availability of the complete 1918 influenza virus coding sequence, we used reverse genetics to generate an influenza virus bearing all eight gene segments of the pandemic virus to study the properties associated with its extraordinary virulence. In stark contrast to contemporary human influenza H1N1 viruses, the 1918 pandemic virus had the ability to replicate in the absence of trypsin, caused death in mice and embryonated chicken eggs, and displayed a high-growth phenotype in human bronchial epithelial cells. Moreover, the coordinated expression of the 1918 virus genes most certainly confers the unique high-virulence phenotype observed with this pandemic virus.

the HA from the Tx/91 virus with the remaining seven genes from the 1918 virus (Tx/91 HA:1918); a virus having the NA from 1918 with the remaining seven genes from the Tx/91 virus (1918 NA:Tx/91); and recombinant viruses having two 1918 (1918 HA/NA:Tx/91) or five 1918 genes (1918 HA/NA/M/NP/NS:Tx/91) with the remaining genes derived from the Tx/91 virus. The HA of the 1918 viruses used throughout these studies was derived from A/South Carolina/1/18 strain that was shown to preferentially bind the α2,6 sialic acid (human) cellular receptor (*16*). The identity of the 1918 and Tx/91 influenza virus genes in the rescued viruses was confirmed by reverse transcription polymerase chain reaction and sequence analysis.

The infectivity of the 1918 virus and the ability to form plaques in the presence and in the absence of the protease trypsin were assayed in MDCK cells by the plaque method. The proteolytic cleavage of the HA molecule is a prerequisite for multicycle replication, and the ability of an influenza virus to replicate in the absence of trypsin has been thought to be an important de-

Few example

2018: Recreating the Horsepox virus

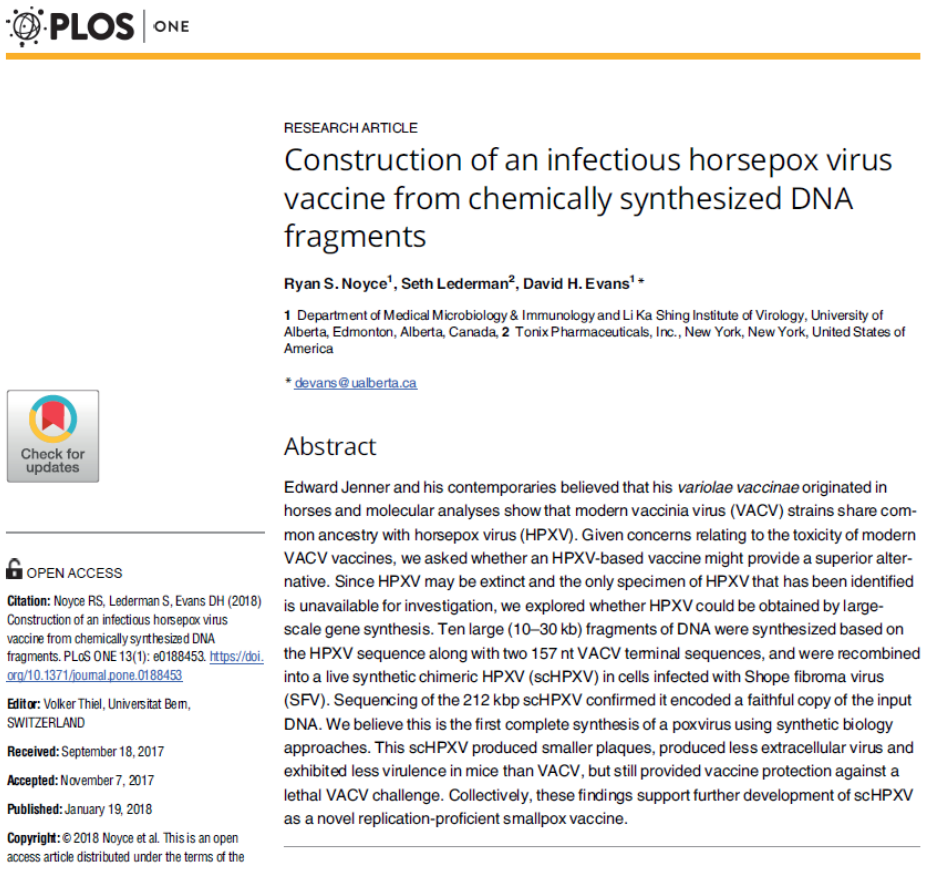
"Ten large fragments of DNA were synthesized based on the HPXV sequence, […] and were recombined into a live synthetic chimeric HPXV. […] We believe this is the first complete synthesis of a poxvirus using synthetic biology approaches."

Scientific justification:

- To find a technical way to develop vaccines against smallpox.

Possible misuse:

- Blueprint to create a deadly pathogen for 100,000$



PLOS ONE

RESEARCH ARTICLE

Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments

Ryan S. Noyce[1], Seth Lederman[2], David H. Evans[1]*

1 Department of Medical Microbiology & Immunology and Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada, **2** Tonix Pharmaceuticals, Inc., New York, New York, United States of America

* devans@ualberta.ca

Check for updates

OPEN ACCESS

Citation: Noyce RS, Lederman S, Evans DH (2018) Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments. PLoS ONE 13(1): e0188453. https://doi.org/10.1371/journal.pone.0188453

Editor: Volker Thiel, Universitat Bern, SWITZERLAND

Received: September 18, 2017

Accepted: November 7, 2017

Published: January 19, 2018

Abstract

Edward Jenner and his contemporaries believed that his *variolae vaccinae* originated in horses and molecular analyses show that modern vaccinia virus (VACV) strains share common ancestry with horsepox virus (HPXV). Given concerns relating to the toxicity of modern VACV vaccines, we asked whether an HPXV-based vaccine might provide a superior alternative. Since HPXV may be extinct and the only specimen of HPXV that has been identified is unavailable for investigation, we explored whether HPXV could be obtained by large-scale gene synthesis. Ten large (10–30 kb) fragments of DNA were synthesized based on the HPXV sequence along with two 157 nt VACV terminal sequences, and were recombined into a live synthetic chimeric HPXV (scHPXV) in cells infected with Shope fibroma virus (SFV). Sequencing of the 212 kbp scHPXV confirmed it encoded a faithful copy of the input DNA. We believe this is the first complete synthesis of a poxvirus using synthetic biology approaches. This scHPXV produced smaller plaques, produced less extracellular virus and exhibited less virulence in mice than VACV, but still provided vaccine protection against a lethal VACV challenge. Collectively, these findings support further development of scHPXV as a novel replication-proficient smallpox vaccine.

Loss of Function and Gain of Function

Loss-of-function: to genetically alters an organism in a way that may reduce partially or completely the biological functions of gene products, usually inducing a reduced pathogenesis, transmissibility, etc. of a pathogen.

Gain-of-function: to genetically alters an organism in a way that may enhance the biological functions of gene products inducing a increased pathogenesis, transmissibility, etc. of a pathogen.

This research is intended to reveal targets to better predict emerging infectious diseases and to develop vaccines and therapeutics.

Few example

2012: Aerosolization of H5N1

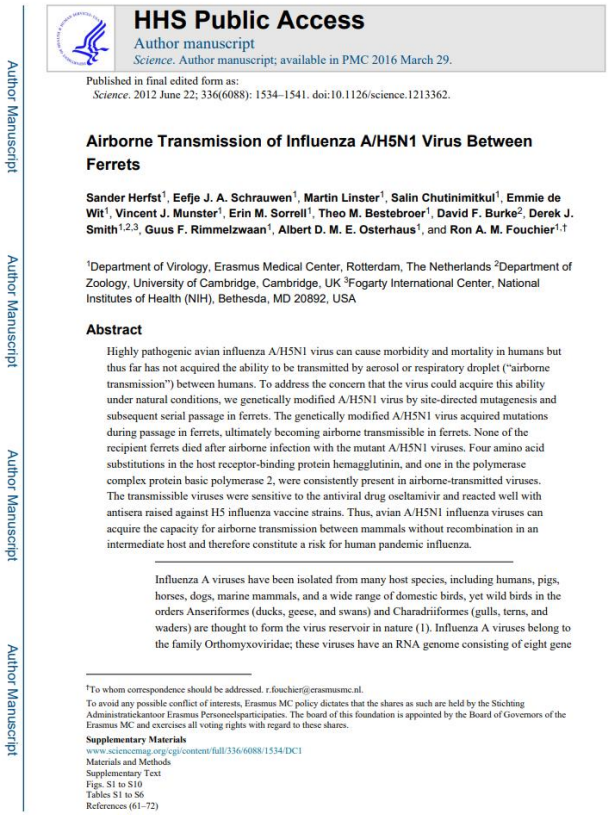
"we genetically modified A/H5N1 virus by site-directed mutagenesis and subsequent serial passage in ferrets. The genetically modified A/H5N1 virus acquired mutations during passage in ferrets, ultimately becoming airborne transmissible in ferrets."

Scientific justification:

- To understand the mechanisms of aerosolization and mammals-to-mammals transmission.
- To better survey the emergence of transmissible H5N1 viruses in humans.

Possible misuse:

- Blueprint to create/weaponize a deadly pathogen.



Author Manuscript Author Manuscript Author Manuscript Author Manuscript

HHS Public Access

Author manuscript

Science. Author manuscript; available in PMC 2016 March 29.

Published in final edited form as:
Science. 2012 June 22; 336(6088): 1534–1541. doi:10.1126/science.1213362.

Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets

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

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

Abstract

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 Charadriiformes (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

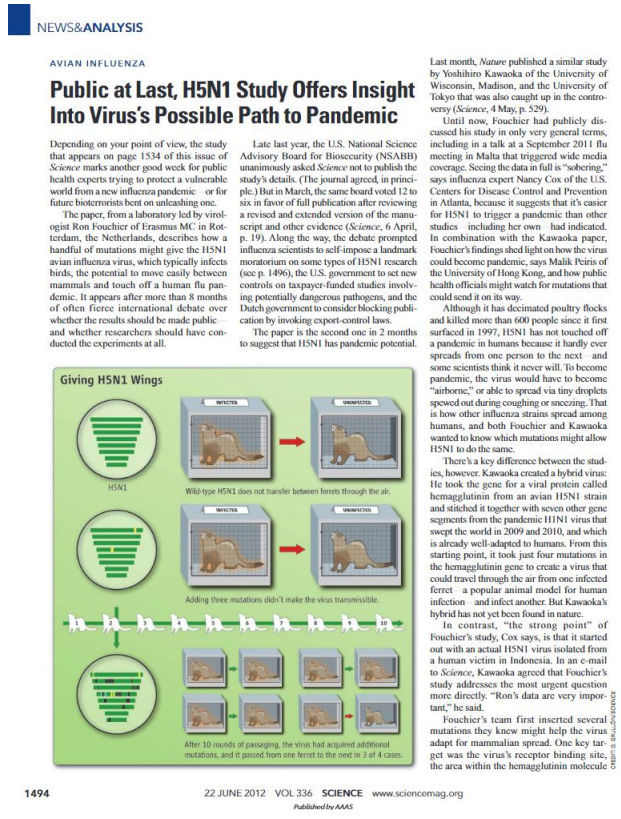
†To whom correspondence should be addressed. r.fouchier@erasmusmc.nl.

To avoid any possible conflict of interests, Erasmus MC policy dictates that the shares as such are held by the Stichting Administratiekantoor Erasmus Personeelsparticipaties. The board of this foundation is appointed by the Board of Governors of the Erasmus MC and exercises all voting rights with regard to these shares.

Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6088/1534/DC1

Materials and Methods
Supplementary Text
Figs. S1 to S10
Tables S1 to S6
References (61–72)



NEWS&ANALYSIS

AVIAN INFLUENZA

Public at Last, H5N1 Study Offers Insight Into Virus's Possible Path to Pandemic

Depending on your point of view, the study that appears on page 1534 of this issue of *Science* marks another good week for public health experts trying to protect a vulnerable world from a new influenza pandemic – or for future bioterrorists bent on unleashing one.

The paper, from a laboratory led by virologist Ron Fouchier of Erasmus MC in Rotterdam, the Netherlands, describes how a handful of mutations might give the H5N1 avian influenza virus, which typically infects birds, the potential to move easily between mammals and touch off a human flu pandemic. It appears after more than 8 months of often fierce international debate over whether the results should be made public and whether researchers should have conducted the experiments at all.

Late last year, the U.S. National Science Advisory Board for Biosecurity (NSABB) unanimously asked *Science* not to publish the study's details. (The journal agreed, in principle.) But in March, the same board voted 12 to six in favor of full publication after reviewing a revised and extended version of the manuscript and other evidence (*Science*, 6 April, p. 19). Along the way, the debate prompted influenza scientists to self-impose a landmark moratorium on some types of H5N1 research (see p. 1496), the U.S. government to set new controls on taxpayer-funded studies involving potentially dangerous pathogens, and the Dutch government to consider blocking publication by invoking export-control laws.

The paper is the second one in 2 months to suggest that H5N1 has pandemic potential.

Last month, *Nature* published a similar study by Yoshihiro Kawaoka of the University of Wisconsin, Madison, and the University of Tokyo that was also caught up in the controversy (*Science*, 4 May, p. 529).

Until now, Fouchier had publicly discussed his study in only very general terms, including in a talk at a September 2011 flu meeting in Malta that triggered wide media coverage. Seeing the data in full is "sobering," says influenza expert Nancy Cox of the U.S. Centers for Disease Control and Prevention in Atlanta, because it suggests that it's easier for H5N1 to trigger a pandemic than other studies – including her own – had indicated. In combination with the Kawaoka paper, Fouchier's findings shed light on how the virus could become pandemic, says Malik Peiris of the University of Hong Kong, and how public health officials might watch for mutations that could send it on its way.

Although it has decimated poultry flocks and killed more than 600 people since it first surfaced in 1997, H5N1 has not touched off a pandemic in humans because it hardly ever spreads from one person to the next – and some scientists think it never will. To become pandemic, the virus would have to become "airborne," or able to spread via tiny droplets spewed out during coughing or sneezing. That is how other influenza strains spread among humans, and both Fouchier and Kawaoka wanted to know which mutations might allow H5N1 to do the same.

There's a key difference between the studies, however. Kawaoka created a hybrid virus: He took the gene for a viral protein called hemagglutinin from an avian H5N1 strain and stitched it together with seven other gene segments from the pandemic H1N1 virus that swept the world in 2009 and 2010, and which is already well-adapted to humans. From this starting point, it took just four mutations in the hemagglutinin gene to create a virus that could travel through the air from one infected ferret – a popular animal model for human infection – and infect another. But Kawaoka's hybrid has not yet been found in nature.

In contrast, "the strong point" of Fouchier's study, Cox says, is that it started out with an actual H5N1 virus isolated from a human victim in Indonesia. In an e-mail to *Science*, Kawaoka agreed that Fouchier's study addresses the most urgent question more directly. "Ron's data are very important," he said.

Fouchier's team first inserted several mutations they knew might help the virus adapt for mammalian spread. One key target was the virus's receptor binding site, the area within the hemagglutinin molecule

Giving H5N1 Wings

H5N1

Wild-type H5N1 does not transfer between ferrets through the air.

Adding three mutations didn't make the virus transmissible.

After 10 rounds of passaging, the virus had acquired additional mutations, and it passed from one ferret to the next in 3 of 4 cases.

CREDIT: D. BRILLIANT/SCIENCE

New risks

Technological Convergences:

- Nanotechnologies, biotechnologies, Whole genome sequencing, informatics, AI,…

Synthetic Biology:

- CrispR cas9; nucleic ac. biosynthesis,…

« **Do it Yourself » science**

Open science:

- # of genomes available, processes & methods freely available,…

New risks

2018: Creation of Synthetic Life

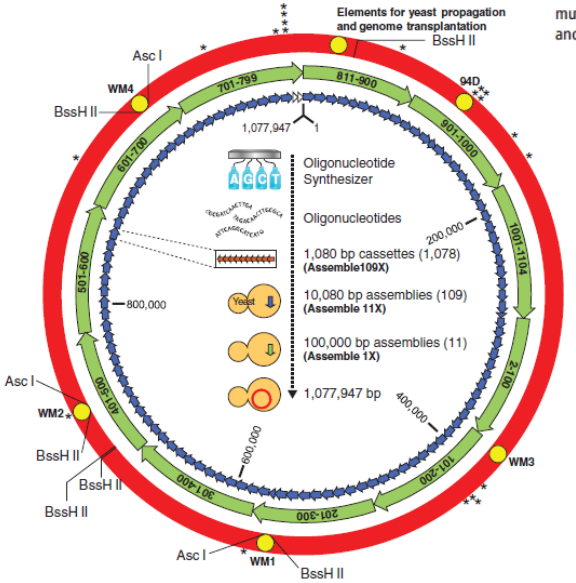
"We report the design, synthesis, and assembly of the 1.08–mega–base pair Mycoplasma mycoides genome starting from digitized genome sequence information […] to create new M. mycoides cells that are controlled only by the synthetic chromosome. […] The new cells have expected phenotypic properties and are capable of continuous self-replication.."

Scientific justification:

- Technological challenge.
- The origin of life

Possible misuse:

- Limitless synthetic possibilities



RESEARCH ARTICLE

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*

We report the design, synthesis, and assembly of the 1.08–mega–base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

How to avoid/mitigate DURC ?

Benefit

Scientific legitimacy

Ethic

Risk of misuse

Gravity of misuse

Accessibility and easiness

Personal and collegial

QUESTIONNAIRE: Research

QUESTIONS	YES	NO	Unsure
Could the project/the task(s) result in **increasing the pathogenicity or virulence** of an infectious agent or the toxicity of a toxin?			
Could the project **generate an agent able to lower immunity to or reduce the efficacy of immunisation** against an infectious agent or toxin on a long-term basis or even permanently?			
Does(Do) the project/the task(s) confer **resistance on a biological agent or toxin** (or toxic agent) to any of the prevention or treatment methods used against the agent?			
Could the project/the task(s) result in making it easier for biological agents or toxins (or toxic agents) to **avoid detection** by biological diagnosis methods or detection methods?			
Could the project/the task(s) result in **increasing the environmental stability, carriage, contagiousness or spread** of a biological agent or toxin (or toxic agent)?			
Could the project generate an agent able to **reduce the sensitivity or resistance of a host or population** to an infectious agent or toxin on a long-term basis or even permanently?			
Could the project/ the task(s) result in **a long-term or permanent change in the tissue and/or cellular target or tropism of an infectious agent**, toxin or toxic agent, thus heightening its pathological properties or giving it new ones?			
Could the project result in generating a biological agent that would **accelerate a disease or degenerative process**?			
Could the project result in the **creation of a new infectious agent with unknown properties or a previously eradicated or extinct infectious agent**, or in the synthesis of a new toxin?			

QUESTIONNAIRE: Research

If "Yes":

QUESTIONS	YES	NO
Is there a knowledge or application driver which **justifies the project from the scientific, clinical or therapeutic point of view**? (you must state your reasoning)?		
Reasons: …… …… ……		
Has a risk assessment been performed on the project in accordance with the **WHO 'Biorisk management: Laboratory biosecurity guidance**'? http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf		

If so, please give details (when, findings, etc.)

……
……
……

QUESTIONNAIRE: Publication

QUESTIONS	YES	NO
Did you discuss the potential dual use of the content of the paper **before publication**?		
If YES, what were the key points of your discussion: – The material of concern?		
– The **potential dual use of the information** (presentation and objective of research, material and methods, results, discussion of results)?		
– The need to include **contextual information justifying the publication** (significance of the research findings, usefulness of the information of technology to the scientific community…)?		
– The need to **introduce modifications** (e.g. de-coupling the material of concern from some or all of the potentially useful scientific information)?		
– The timing/process of publication: do you consider that your publication could be improved on the question of potential dual use concern by recommendations from the DULG and/or the Ethics Board?		
– The access to be given to the content of the publication, in order to decide if : – there was or not specific limit to distribution? – access must be limited or not on a “need-to-know” basis?		
– Other issues : ……		
If NO, please explain the reason(s) why you considered that the question related to “potential dual use” was not applicable to the content of your publication or needed to be discussed? ……		

You are the Dual-Use committee:

2002:

REPORTS

Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural Template

Jeronimo Cello, Aniko V. Paul, Eckard Wimmer*

Full-length poliovirus complementary DNA (cDNA) was synthesized by assembling oligonucleotides of plus and minus strand polarity. The synthetic poliovirus cDNA was transcribed by RNA polymerase into viral RNA, which translated and replicated in a cell-free extract, resulting in the de novo synthesis of infectious poliovirus. Experiments in tissue culture using neutralizing antibodies and CD155 receptor–specific antibodies and neurovirulence tests in *CD155* transgenic mice confirmed that the synthetic virus had biochemical and pathogenic characteristics of poliovirus. Our results show that it is possible to synthesize an infectious agent by in vitro chemical-biochemical means solely by following instructions from a written sequence.

- Synthesis of a full-length poliovirus complementary DNA (cDNA) by combining overlapping segments of 400 to 600 base pairs (bp).
- cDNA was transcribed by RNA polymerase into viral RNA using cell extracts.
- Transfer into cells resulted in replication and de novo synthesis of infectious poliovirus.
- Cell culture and sensitive mice experiment demonstrated the virulence of the newly created virus.

Scientific justification:

- Demonstration of the possibility to synthesize an infectious agent by in vitro chemical-biochemical means solely by following instructions from a written sequence.

Possible misuse:

- Availability of a deadly pathogen
- Blueprint to create a deadly pathogen

You are the Dual-Use committee:

2001:

JOURNAL OF VIROLOGY, Feb. 2001, p. 1205–1210 Vol. 75, No. 3
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.3.1205–1210.2001

Expression of Mouse Interleukin-4 by a Recombinant Ectromelia Virus Suppresses Cytolytic Lymphocyte Responses and Overcomes Genetic Resistance to Mousepox

RONALD J. JACKSON,[1,2]* ALISTAIR J. RAMSAY,[2]† CARINA D. CHRISTENSEN,[2] SANDRA BEATON,[1] DIANA F. HALL,[1]‡ AND IAN A. RAMSHAW[2]

Pest Animal Control Cooperative Research Centre, CSIRO Sustainable Ecosystems,[1] and Division of Immunology and Cell Biology, John Curtin School of Medical Research, Australian National University,[2] Canberra, Australia

Received 25 July 2000/Accepted 13 November 2000

Genetic resistance to clinical mousepox (ectromelia virus) varies among inbred laboratory mice and is characterized by an effective natural killer (NK) response and the early onset of a strong $CD8^+$ cytotoxic T-lymphocyte (CTL) response in resistant mice. We have investigated the influence of virus-expressed mouse interleukin-4 (IL-4) on the cell-mediated response during infection. It was observed that expression of IL-4 by a thymidine kinase-positive ectromelia virus suppressed cytolytic responses of NK and CTL and the expression of gamma interferon by the latter. Genetically resistant mice infected with the IL-4-expressing virus developed symptoms of acute mousepox accompanied by high mortality, similar to the disease seen when genetically sensitive mice are infected with the virulent Moscow strain. Strikingly, infection of recently immunized genetically resistant mice with the virus expressing IL-4 also resulted in significant mortality due to fulminant mousepox. These data therefore suggest that virus-encoded IL-4 not only suppresses primary antiviral cell-mediated immune responses but also can inhibit the expression of immune memory responses.

- Genetic comparison between resistant and sensitive mouse to mousepox -> mediated by NK cell and cytotoxic T-lymphocyte (CTL)
- Interleukin 4 (Il-4) mitigate the inflammation.
- Engineering of Il-4 expressing Mousepox virus.
- Il-4 expressing Mousepox counterpass the natural resistance of mice
- Enhance virulence and mortality of the virus.

Scientific justification:

- To understand the mechanisms of resistance against a poxvirus.
- To better fight fatal infection with medical countermesure.

Possible misuse:

- Engineering of a deadly pathogen
- Blueprint to create an enhanced deadly pathogen

You are the Dual-Use committee:

2021:

CORONAVIRUS

Prospective mapping of viral mutations that escape antibodies used to treat COVID-19

Tyler N. Starr[1]*, Allison J. Greaney[1,2,3]*, Amin Addetia[1,4], William W. Hannon[1,4], Manish C. Choudhary[5], Adam S. Dingens[1], Jonathan Z. Li[5], Jesse D. Bloom[1,2,6]†

Antibodies are a potential therapy for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), but the risk of the virus evolving to escape them remains unclear. Here we map how all mutations to the receptor binding domain (RBD) of SARS-CoV-2 affect binding by the antibodies in the REGN-COV2 cocktail and the antibody LY-CoV016. These complete maps uncover a single amino acid mutation that fully escapes the REGN-COV2 cocktail, which consists of two antibodies, REGN10933 and REGN10987, targeting distinct structural epitopes. The maps also identify viral mutations that are selected in a persistently infected patient treated with REGN-COV2 and during in vitro viral escape selections. Finally, the maps reveal that mutations escaping the individual antibodies are already present in circulating SARS-CoV-2 strains. These complete escape maps enable interpretation of the consequences of mutations observed during viral surveillance.

- Prospective mapping of all AA site on the Spike protein involved in:
- Recognized by therapeutic Antibodies
- patients with persistent infection
- Important for immune escape

Scientific justification:

- To understand the mechanisms of immune escape.
- To anticipate emergence of variant.
- To adapt the countermeasure by anticipation.

Possible misuse:

- Mapping for enhanced viruses

You are the Dual-Use committee:

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Engineering SARS-CoV-2 using a reverse genetic system

Xuping Xie[#1], Kumari G. Lokugamage[#2], Xianwen Zhang[#1], Michelle N. Vu[2], Antonio E. Muruato[1,2], Vineet D. Menachery[2,3,4,*], Pei-Yong Shi[1,3,5,6,7,*]

Abstract

Reverse genetic systems are a critical tool for studying viruses and identifying countermeasures. In response to the ongoing pandemic of COVID-19, we recently developed an infectious cDNA clone for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The reverse genetic system can be used to rapidly engineer viruses with desired mutations to study the virus *in vitro* and *in vivo*. Viruses can also be designed for live-attenuated vaccine development and engineered with reporter genes to facilitate sero-diagnosis, vaccine evaluation, and antiviral screening. Thus, the reverse genetic system of SARS-CoV-2 will be widely used for both basic and translational research. However, due to the large size of coronavirus genome (~30,000 nucleotides long) and several toxic genomic elements, manipulation of the reverse genetic system of SARS-COV-2 is not

- describe the technical details of how to engineer recombinant SARS-CoV-2.

Scientific justification:

- studying viruses and identifying countermeasures:
  - live-attenuated vaccine, facilitate sero-diagnosis, vaccine evaluation, and antiviral screening.
- Accelerate research on SARS-CoV-2.

Possible misuse:

- Blueprint to create any variant for SARS-CoV-2 and other viruses.

You are the Dual-Use committee:

PNAS

Analyzing a bioterror attack on the food supply: The case of botulinum toxin in milk

Lawrence M. Wein*† and Yifan Liu‡

*Graduate School of Business and ‡Institute for Computational and Mathematical Engineering, Stanford University, Stanford, CA 94305

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We developed a mathematical model of a cows-to-consumers supply chain associated with a single milk-processing facility that is the victim of a deliberate release of botulinum toxin. Because centralized storage and processing lead to substantial dilution of the toxin, a minimum amount of toxin is required for the release to do damage. Irreducible uncertainties regarding the dose–response curve prevent us from quantifying the minimum effective release. However, if terrorists can obtain enough toxin, and this may well be possible, then rapid distribution and consumption result in several hundred thousand poisoned individuals if detection from early symptomatics is not timely. Timely and specific in-process testing has the potential to eliminate the threat of this scenario at a cost of <1 cent per gallon and should be pursued aggressively. Investigation of improving the toxin inactivation rate of heat pasteurization without sacrificing taste or nutrition is warranted.

bioterrorism | mathematical modeling

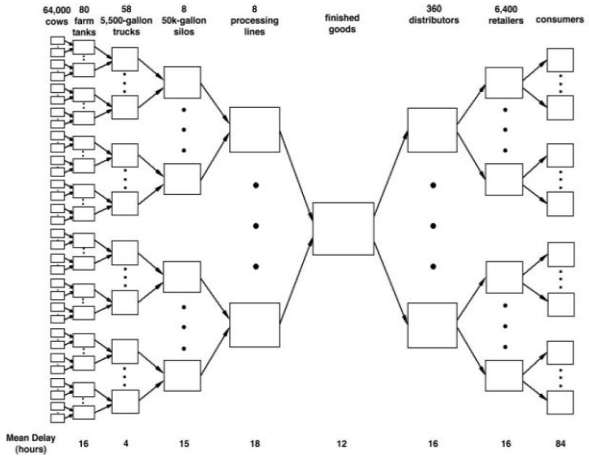

Fig. 1. The milk supply chain.

- Mathematic model to create and analyse a bioterror attack of large scale on food-chain supply:
  - What to attack
  - Where to attack
  - What amount and concentration of toxin is necessary
  - Evaluation of casualties

Scientific justification:

- Studying weaknesses of the system.
- Anticipate risks and consequences.
- Prevent an attack with surveillance.

Possible misuse:

- Thanks, it's a good idea!

You are the Dual-Use committee:

- **Do you have an example of potential Dual-use research ?**

Acknowledgement

The **European Union's Chemical, Biological, Radiological and Nuclear risk mitigation Centres of Excellence Initiative (EU CBRN CoE)**.

➡ addresses the **mitigation** of and **preparedness** against biological and biosafety-related risks

Strengthen regional Health security