Policy Case Study: Synthesis of the Horsepox Virus

from

ROADMAP FOR IMPEMENTING BIOSECURITY AND BIODEFENSE POLICY IN THE UNITED STATES

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Summary

In 2016, Canadian researchers informed the World Health Organization Advisory Committee on Variola Virus Research that they had synthetized horsepox virus, a previously extinct orthopoxvirus, in 6 months and with only $100,000.(2) These claims elicited concern about dual use potential of the research among the WHO committee members and subsequently, among biosecurity experts in the United States. Among their concerns are the risks that publication of the methods could enable a malicious actor to replicate the research with smallpox or another harmful orthopoxvirus, and that scientists, either knowingly or unknowingly, could assist a terrorist group in creating smallpox.(3, 4) In addition, the researchers describe three reasons for conducting this research: 1) to show that synthesis of an orthopoxvirus could be done; 2) to create a viral vector that can attack cancerous cells; and 3) to be used as a potential candidate vaccine for smallpox. The researchers' claims, the biosecurity community's concerns, and the stated reasons for conducting this type of research provide a useful case study with which to examine the broader policy implications of synthesis of an orthopoxvirus. In this case study, we review the actual experiments involved in the research and the regulatory environment in which it was conducted, evaluate the policy and scientific enablers, and explore the relevance of existing U.S. policy on similar types of research if it were conducted in the U.S.

The key findings and conclusions from this case study are:

- The existing regulatory system for governing life sciences research in the United States is overlapping and if implemented well, could result in review and oversight of research involving synthesis of an extinct pathogen and an orthopoxvirus.

- If followed exactly as written, biosecurity policies would not apply to synthesis of horsepox virus. However, biosafety and ethics policies likely would trigger review and oversight of such research even though security experts did not raise these concerns.
  - The Institutional Biosafety Committee would review the research for risks of accidental exposure (i.e., biosafety risks) to comply with the NIH guidelines for recombinant and synthetic nucleic acids, and of dual use if its review processes exceed the federal policy on dual use life sciences research of concern.
  - The biosafety official would conduct a risk assessment for biosafety and biosecurity, and identify risk mitigation strategies to comply with the 5th Edition of the Biosafety in Microbiological and Biomedical Research Laboratories Manual.

- From a scientific standpoint, the synthesis of horsepox virus requires advanced knowledge and skill suggesting that well-resourced actors who have existing poxvirus research capabilities may be able to reproduce the research.

- Because vaccinia virus (the original smallpox vaccine) is 98% identical to horsepox virus and may have been derived from horsepox virus, the safety and security risks may be no greater than corresponding risks of vaccinia virus.

- From an international perspective, scientific and national differences in understanding and addressing dual use life sciences research present significant challenges in promulgating practices that could help identify and mitigate serious biosecurity risks.

The Policy Backdrop

After the 2001 terrorist attacks, the United States (U.S.) invested billions of dollars in research and development of medical countermeasures (MCM) (specifically, vaccines and drugs) against material biological, chemical, and radiological threats. These investments provide funding for activities at all research, development, and approval steps of the MCM development pipeline.

Basic research efforts involve a variety of studies in cultured cells and animals to identify which parts of a pathogen elicit protective immune response, create and test candidate vaccines and drugs, and develop new platform technologies for MCM such as new viral vectors or synthetic organisms that produce therapeutic molecules. MCM that show promise in animals must go through a lengthy process for gaining regulatory approval that is designed to assess the products' safety and effectiveness in humans. For vaccine and drug candidates against common infectious diseases (e.g., malaria and tuberculosis), large numbers of people already are infected or at risk of infection, allowing scientific entities (academic centers, government laboratories, pharmaceutical companies) to recruit hundreds to thousands of people to test the candidate MCM. However, some material threat agents may cause disease sporadically, while others may have been eradicated in nature, making traditional clinical trials difficult or impossible in human populations. Furthermore, natural infection may result in different disease presentation and outcomes than man-made events, such as purposeful release of a material threat agent (i.e., biological, chemical, or radiological agent) or accidental release of a laboratory-made pathogen. The only way to generate the efficacy data for MCMs under the typical vaccine or drug approval process would be to expose human subjects to the agent,(1) which for many material threat agents is considered unethical.(2)

Because generating the data on how well the candidate vaccine or drug works against the material threat agents is a critical step in the approval process, the Food and Drug Administration (FDA) established the FDA Animal Efficacy Rule in 2002. This Rule applies to any candidate vaccine or drug for which human efficacy testing is either unethical or infeasible. The 2015 guidance related to MCM development is most relevant to this case study. This Rule and associated guidance allows the FDA to use data from animal studies, in lieu of human trials, to evaluate the effectiveness of the candidate MCM against the relevant material threat agent(s). The primary challenge in using this Rule for approval is the development of animal models that reflect human infection and disease with relevant material threat agents and routes of exposure. To generate the scientific data needed for this work, the FDA and U.S. National Institutes of Health formalized a partnership in 2010 to fund research in regulatory science to enable testing

of the efficacy of candidate MCMs and pharmaceutical products against other rare diseases.

In addition to these efforts, the U.S. Congress passed laws to incentivize scientific entities to develop MCMs. These incentives included the establishment of milestone-based payments for interim results of candidate products, formation of the Biomedical Advanced Research and Development Authority (BARDA) to fund advanced development of MCM, and the creation of the Emergency Use Authorization (EUA) to allow MCMs within 8 years of FDA approval to be procured by the U.S. Strategic National Stockpile (the U.S. repository of critical medicines for emergencies). Most recently, Congress passed the 21st Century Cures Act, which includes provisions for priority review vouchers for candidate MCMs. Through this program, the FDA may provide priority review vouchers for products meeting certain criteria after approval of a material threat MCM application. The priority review voucher can be used by the recipient or sold or transferred to another organization who may use the voucher for a product that would not otherwise receive priority review. This program incentivizes companies to develop MCMs against material threats (for which commercial markets do not exist) by providing opportunities to buy down the financial risks of product development for both MCM and other FDA-regulated pharmaceuticals.

In 2004, the U.S. Congress passed the Intelligence Reform and Terrorism Prevention Act, which includes a provision stating that to "knowingly produce, engineer, synthesize, acquire, transfer directly or indirectly, receive, possess, import, export, or use, or possess and threaten to use, variola virus" is unlawful.(3) This section defines variola virus as "a virus that can cause human smallpox or any derivative of the variola major virus that contains more than 85% of the gene sequence of the variola major virus or the variola minor virus."(3) This law caused significant concern among poxvirus researchers in the U.S. about the risk of criminal charges being brought against researchers working with poxviruses because most share greater than 85% sequence similarity to variola virus (also called smallpox virus). In 2006, an international group of researchers published the genomic sequence of a 1976 isolate of horsepox virus and showed its relationship to vaccinia virus and other members of the orthopoxvirus family, including the smallpox virus.(4) The protein sequence derived from the horsepox virus genome is 98% identical to vaccinia virus, which is the historical vaccine for smallpox. The authors describe genetic sequences that are shared between the smallpox virus and horsepox, but they do not describe the overall percent identity between the viruses. Although horsepox virus is thought to be extinct, some scientists believe that vaccinia virus, the original smallpox vaccine, was derived horsepox and originally came from poxvirus infections in horses.(5, 6)

As these efforts evolved, the U.S. government examined the potential for harmful use of legitimate research involving pathogens. These efforts, which fall under the dual use research of concern umbrella, informed the development of U.S. policies on review and oversight of such research. Although the initial policy dialogues focused on pathogen research that could result in certain traits or create extinct pathogens, as described in the National Research Council Report *Biotechnology Research in an Age of Terrorism*

and documents from the National Science Advisory Board for Biosecurity, the federal policies ultimately covered research with certain traits of concern in 15 specified pathogens. One of these pathogens is smallpox and one of the traits of concern is resurrection of an extinct pathogen or toxin. In 2017, the U.S. government issued additional guidance for dual use research of concern (Recommended Policy Guidance for Departmental Development of Review Mechanisms for Potential Pandemic Pathogen Care and Oversight (P3CO)), which currently is being implemented by federal agencies that fund life sciences research. This new guidance adds to the current policy on dual use life sciences of concern by adding a new category of restrictions – specifically on research with pathogens that could cause a human pandemic if released from laboratories – and instructing federal agencies to develop new procedures for reviewing and overseeing such research.

The Horsepox Virus Synthesis and Regulatory Context

The Experiment

In November 2016, Dr. David Evans, a vaccinia virus researcher at the University of Alberta in Edmonton, Canada, discussed his laboratory's recent achievement in synthesizing horsepox virus with the World Health Organization (WHO) Advisory Committee on Variola Virus Research, of which he is a member.(7, 8) The horsepox virus genome is 212 kilobases, has complex structures at its ends, and was described in 2006. Dr. Evans' laboratory purchased overlapping DNA fragments, each about 30kb long, that spanned the entire genomic sequence of horsepox virus from a commercial vendor. The researchers purchased 157 base pair long DNA fragments corresponding to the vaccinia virus end segments from Integrated DNA Technologies.(7) The researchers connected the purchased end segments to the ends of the purchased DNA and introduced those DNA fragments into cells that were infected with an animal virus in the poxvirus family(9-11) (a Leporipoxvirus), which resulted in the creation of infectious horsepox virus.(12). Figure 1 shows a schematic of the horsepox synthesis experiment, based on the 2018 publication of the research.(7)

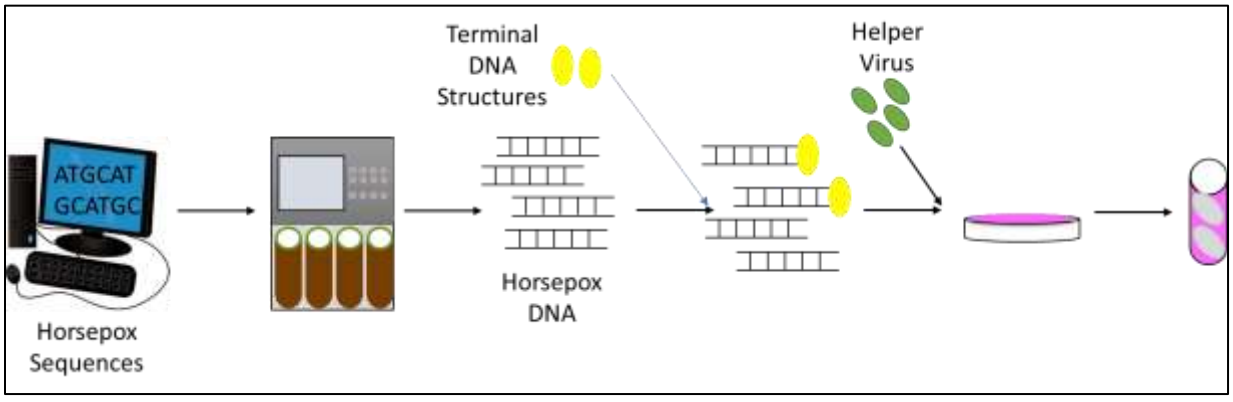

Figure 17. Schematic of the expected experimental procedure used to create horsepox virus from sequence.

The Evans laboratory claims to have spent $100,000 and 6 months synthesizing and recovering infectious horsepox virus. However, their experimental procedures appear to

have been developed and optimized well before 2016. A review of Dr. Evans' publication record highlights his previous efforts in developing and optimizing the experimental procedures he used for the synthesis of horsepox virus, a conclusion supported by the discussion of the WHO advisory group. Furthermore, this timeframe does not account for the scientific knowledge, skill, materials, and poxvirus parts that previously existed in the Evans Laboratory. In addition, the stated timeframe does not include the years of research involved in defining the optimal sequence lengths and terminal pieces that were needed to gain full coverage of the genome and to add the termini to the fragments. Therefore, the level of tacit knowledge needed to create horsepox virus from published sequence was high, requiring specialized skill and knowledge which many actors do not have.

Canada's Biosafety and Biosecurity Policy Framework

According to the publication, Dr. Evans contacted the relevant Canadian regulatory authorities to seek approval for the research.(7) In 2009, Canada passed the Human Pathogens and Toxins Act (HPTA), which establishes a safety and security regime for human pathogens and toxins that pose significant risks to public health and safety.(13) On December 1, 2015, the HPTA and the *Human Pathogens and Toxins Regulations* (HPTR) were fully implemented, which allows for oversight for activities including the import, export, handling, production, permitting access to, possession, use, storage, release, disposal, or transfer of human pathogens and toxins. The scope of the HPTA includes all Risk Group 2 to 4 human pathogens and toxins, whether imported or domestically acquired, or naturally occurring or synthesized.(14)

The HPTA requires facilities to obtain a license for activities with Risk Groups 2, 3, and 4 human pathogens and toxins, (equivalent to biosafety levels 2, 3, and 4 in the United States), and reinforces institutions' internal accountability systems. The Canadian Biosafety Standard (CBS) is a national standard that sets out the physical containment, operational practice, and performance and verification testing requirements for the safe handling and storing of human and terrestrial animal pathogens and toxins in Canada.(15) The CBS is used by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) to verify the ongoing compliance of facilities regulated under the HPTA, and the Health of Animals Act and *Health of Animals Regulations* (HAR) to support license applications and renewals for human pathogens and toxins, and animal pathogen import permits.

Under the HPTA, PHAC delivers a national program that includes individual security clearances for those with access to select high risk pathogens, laboratory incident reporting, compliance promotion, monitoring and verification, pathogen risk assessments, standards and guidance development, biosafety and biosecurity awareness and training, stakeholder engagement, and enforcement.(16)

The HPTR also require facilities conducting scientific research to develop and submit a plan for administrative oversight that describes how their facility administratively manages and controls biosafety and biosecurity risks at the institutional level, including the identification, assessment and mitigation of risks associated with research with

dual-use potential.(17) Ongoing compliance monitoring activities conducted by PHAC verify that regulated facilities are adhering to appropriate biosafety and biosecurity practices, including those described in their plans for administrative oversight. Dr. Evans' institution, the University of Alberta, submitted a plan for administrative oversight to PHAC as part of the University's HPTA license application.(16, 18, 19)

Prior to the full implementation of the HPTA and HPTR, PHAC worked with the regulated community to guide them through the implementation transition period and to provide them with resources to help them comply.

The Canadian Food Inspection Agency has regulatory authority of pathogens causing foreign animal diseases and pathogens causing emerging animal diseases that are imported into the country under the *Health of Animals Act* and the *Health of Animals Regulations*.

Relevant U.S. Policy Considerations

Dr. Evans informed the WHO that he chose to synthesize horsepox virus to show that it was feasible with publicly available information and relatively few funds and time.(8) However, all other publicly-available articles describe this re-created virus as an alternative smallpox vaccine. In March 2017, a U.S.-based company, Tonix Pharmaceuticals Holding Corp., issued a press release announcing its partnership with Dr. Evans in developing a new candidate smallpox vaccine.(20) This candidate vaccine is a "live form of horsepox virus that has been demonstrated to have protective vaccine activity in mice." The development of a potential MCM (i.e., the chimeric horsepox virus) for smallpox virus, which is a material threat in the United States, allowed Tonix to be eligible for the priority review voucher program that was established for MCMs in the 2016 21st Century Cures Act.(21) Because Tonix is a U.S. based company, the virus would need to be imported into the United States for advanced development and manufacturing. Transferring the synthesized horsepox virus to the U.S. likely would be regulated by U.S. import regulations for infectious biological agents, infectious substances, and vectors (42 §71.54). However, horsepox virus is not a listed agent on the U.S. Export Administration Regulations Commerce Control List, and if used as a vaccine, the synthesized virus may be excluded from export control regulations if it were listed (ECCN 1C351). The horsepox virus is not listed as a controlled agent by the Australia Group, of which the United States and Canada are members.

An Assessment of U.S. Policy Relevance if the Horsepox Virus was Synthesized in the U.S.

If a research group in the United States attempted to synthesize horsepox virus, the research would not necessarily be restricted within current regulatory and policy frameworks.

- The National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acids would hold U.S. universities responsible for reviewing the proposed research for biosafety. At this stage of review, the Institutional Biosafety Committee (IBC) and/or biosafety official would assess the biosafety risks of the

research (including risks associated with animal studies), recommending research conditions under which the research could be conducted safely. If risks cannot be addressed adequately, they may not approve the research to continue. Adherence to the NIH Guidelines is mandatory for federally-funded research and institutions receiving federal funds, but voluntary for research institutions that do not receive U.S. government research funding.

- The IBCs and biosafety officials would review the research for potential biosecurity risks, per the 5th Edition of the Manual on Biosafety in Microbiological and Biomedical Laboratories (BMBL). Although not required by federal law, most research institutions would comply with the biosafety and biosecurity guidance in the BMBL to promote good practice, comply with funding award requirements, and/or prevent reputational harm, financial penalties, or removal of funding if an accidental release occurs. Furthermore, if the research was regulated by the Federal Select Agent Program (i.e., involving synthesis of a regulated poxvirus), the institution would be required to comply with the BMBL and NIH Guidelines.

- The Animal Welfare Act and Animal Welfare Regulations require institutions to review and oversee research involving animals. According to the Tonix Pharmaceutical press release, the synthesized horsepox virus was studied in mice. Although mice used in research laboratories are explicitly excluded in the Regulations, animals that may be used in advanced development of MCM likely are covered. Furthermore, the NIH Public Health Service Policy requires institutional review and oversight of NIH-funded research involving mice. Therefore, testing of the synthesized virus likely would be reviewed by the Institutional Animal Care and Use Committee if the research was funded by NIH and if studies involved animals covered in the Animal Welfare Regulations. At this stage, questions about the source of the virus may have been raised by the responsible veterinarian and committee members.

- In 2010, the U.S. government released its Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA. This framework is voluntary for industry and resembles industry guidance for sequence and customer screening, which is promulgated by the International Gene Synthesis Consortium (IGSC). The U.S. government's and the IGSC screening frameworks are based largely based on the Biological Select Agents and Toxins list, on which smallpox is listed. If a researcher orders the synthetic DNA from a company that follows this guidance, the company may inquire further, decline to fulfill the order, or contact the Federal Bureau of Investigation or similar governmental authorities given the high degree of similarity between horsepox virus genes and smallpox virus genes. Because smallpox is a restricted agent in the United States and by the World Health Organization, the IGSC companies would treat any orders containing any sequences identical to the smallpox genome differently than other sequences. The company's scrutiny of the order may delay or prevent the research from continuing. However, if the customer has demonstrated its legitimacy, the order may be fulfilled. According to the WHO

report, obtaining the synthetic DNA fragments was the longest step in the synthesis process, but no additional details are provided.

- The horsepox virus is not listed as a Biological Select Agent and Toxin and consequently, does not fall under oversight of the Federal Select Agent Programs. (42 §73 and 9§121)

- The horsepox virus is not one the biological agents listed in the United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern and the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern. However, institutions that use an approach for reviewing dual use potential of life science research that is broader than the current federal policy may recognize the potential security risks of the research, recommend risk reduction strategies for the research, and/or oversee the research.

- The 2017 Recommended Policy Guidance for Departmental Development of Review Mechanisms for Potential Pandemic Pathogen Care and Oversight (P3CO) likely would not enable review and oversight of the horsepox virus synthesis research. U.S. government funders of life sciences research currently are developing their review and oversight processes for implementing this federal guidance document. The guidance states that potential pandemic pathogens are highly transmissible in human populations and highly likely to cause significant illness and/or death. The guidance goes on to describe enhanced potential pandemic pathogen as the modification of natural pathogens to increase their ability to spread between people and to cause increased illness and/or death. At the same time, the guidance excludes modifications that are "associated with developing and producing vaccines." If the security concern about horsepox virus is that information about how to synthesize a poxvirus may aid adversaries, the research would not be covered by the processes developed from this guidance. If the concern is that an adversary can use the synthesized horsepox virus to cause harm to individuals, the research likely would not fall under oversight processes because horsepox virus is thought to only infect and cause disease in animals and Tonix claims this virus was developed as a candidate MCM for smallpox.

- Although a high degree of sequence identity exists between horsepox virus and other orthopoxviruses, the virus may or may not be covered under 18 USC §175c. The definition of variola virus included in the U.S. Code is highly ambiguous, resulting in its clarification by the Department of Justice (DoJ). In 2008, the DoJ defined the term variola virus, within the context of 18 USC §175c, as not including "other naturally occurring orthopoxviruses, such as cowpox and vaccinia, but is rather limited to viruses that cause smallpox or are engineered, synthetized, or otherwise produced by human manipulation from the variola major virus or its components."(22) By this definition, horsepox virus would not be included in this statute, which may be supported by recent publications stating that vaccinia virus is derived from horsepox virus. However, some horsepox genes are identical to smallpox and horsepox previously was considered an extinct virus, raising questions

about whether horsepox may be included. Despite this ambiguity, horsepox probably would not be included in this statute because it was not derived from variola virus.

If the research resulted in a virus that was used to harm humans or animals deliberately, the perpetrator could be prosecuted under the Biological Weapons Anti-Terrorism Act of 1989, which is the United States' implementing legislation for the Biological and Toxins Weapons Convention.

The primary policy findings from this case study are:

1) The U.S. biosecurity policies do not apply to the synthesis of horsepox virus. Detection, regulation, and oversight of research involving synthesis of horsepox virus would not occur through biosecurity policies, including the DNA screening framework guidance, because it is not listed as a Biological Select Agent and Toxin and it is being marketed as a candidate smallpox vaccine.

2) The U.S. biodefense program may provide an incentive and rationale for synthesis of the horsepox virus (i.e., to create a MCM for smallpox). In this situation, the potential benefit of horsepox as a smallpox vaccine has to be compared to the potential risk that knowledge about the methods for synthesizing horsepox virus, especially because MCMs already exist for smallpox virus and vaccinia virus (the original smallpox vaccine) was derived from horsepox virus. Therefore, the publicly-stated benefits *and* risks may be overestimated.

3) The U.S. biosafety guidance and animal care and use requirements, which addresses an ethical risk (protection of research animals), likely would trigger review and oversight of the horsepox virus research.

4) Based on the current regulatory requirements for biosafety and ethics, the system under which life sciences research is conducted, if implemented well, is able to detect, review, oversee, and manage moderate and high-risk research. However, reliance only on biosecurity policies to detect, review, and oversee research is restricted to a defined list of agents. This difference is exacerbated at institutions that precisely comply with relevant policies, which may result in quick policy "fixes" that may adversely affect some, but not necessarily the most relevant research. However, institutions that implement review and oversight procedures that exceed federal policies may be well-situated to detect and mitigate risk proactively, without preventing the research from being conducted and without eliciting exaggerated policy responses based on alarmist sentiments or scientifically unfounded fears.

5) The synthesis of horsepox virus highlights the international nature of the science and technology landscape. The assessment of relevant U.S. policy actions in response to the synthesis of horsepox virus is a thought exercise designed to identify gaps in policy and policy implementation. However, the actual research was conducted outside the U.S. and the researchers are attempting to publish their work, which would be shared with scientists around the world to enable scientific progress and advancement on beneficial research (e.g., viral platforms for creating vaccines

against infectious disease or cancerous cells, both of which are possible uses of horsepox virus). Internationally, the International Health Regulations and Global Health Security Agenda set competencies for biosafety and biosecurity of diagnostic laboratories, but only the World Health Organization's published guidelines for biosafety, biosecurity, and responsible science applies to research laboratories. International scientific organizations have engaged scientists and other organizations on dual use life sciences research. However, scientific and national differences in understanding and addressing dual use life sciences research present significant challenges in promulgating practices that could help identify and mitigate serious biosecurity risks.

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