A Pox In All Our Houses: Discovering the Origin and Evolution of Smallpox Vaccine

Abstract/Summary

This project will sample biological material and historical artifacts of 18th through early 20th century vaccination practices to identify the DNA of historical Orthopoxviruses. The goal is to create an open source, linked data network mapping the migration and mutation of vaccines worldwide and to illuminate the culture of vaccination protocols. To do this we are seeking samples of vaccination material from geographically diverse localities and across time. We seek museums, archives, medical/academic institutions, historical houses, and any other potential source of historical smallpox vaccination kits/samples to join our project. This white paper includes an introduction, the origin of the project, goals, the institutions/groups involved, the sampling needs and protocols, method of analysis, additional research, collaborator logistics, and a brief conclusion.

Introduction

To date, most of what we know about early vaccines comes from historical records, not actual specimens. Early vaccine material, however—scabs and lymph smears on vaccination tools—survives in medical collections worldwide. Only a few samples have been subjected to genetic analysis. These surviving samples are rare and museums and other collections are naturally reluctant to approve the destructive analysis to obtain genetic information. Our team of international researchers proposes to create a genetic map of the origin and evolution of smallpox vaccines from the 18th to the early 20th centuries using a combination of non-destructive and destructive DNA analysis and historical research.

Recent technological advancements in ancient DNA extraction and analysis have enabled us to reconstruct early pathogen genomes from non-primary biological sources. We can now derive partial or even complete genomes from instruments used in early vaccinations such as knife blades or lancets, bone points, storage tins for scabs, and glass plates that held pox lymph. This can be accomplished by rinsing or swabbing the object with nucleic acid extraction buffers and is non-destructive in nature. No physical part of the object is sacrificed to obtain the DNA. Researchers can obtain viable DNA material from museum collections without harming the objects themselves and museum staff can use the results to interpret early material culture.

The Origin of the Pox Project

In April 2016, the Mütter Museum discovered several 19th-century vaccination kits among a tray of phlebotomy tools.[1] Kits contained a tiny lancet, two square glass plates, and a tin box with a sliding lid. The kits' tin boxes contained crumbled scabs, which had the appearance of tiny fragments of topaz. Recognizing the possible infection risk, the museum contacted the Pennsylvania Department of Health and the Centers for Disease Control and Prevention (CDC). Health officials wanted the specimens, and two days later representatives from the Philadelphia Department of Public Health came to the museum to collect all kits and send them to CDC in Atlanta, Georgia. By the end of April, CDC had assayed the samples and three of the five kits yielded hits for an unknown pox-family virus: scab material in two kits and residue on glass plates in the third. The results "suggest[ed] the presence of Orthopoxvirus DNA in these specimens that is not consistent with *variola* virus or contemporary strains of *vaccinia* virus." *Variola* and *vaccinia* refer to the smallpox virus and the Orthopoxvirus used as a vaccine against smallpox, respectively. If the samples do not indicate *variola* or current-day *vaccinia*, then what could be the source? The samples may represent an older *vaccinia* virus that assays cannot currently detect, or another Orthopoxvirus, possibly one that infected horses (horsepox) or another mammal. The only way to be certain was to culture the specimens for further analysis.

By August 2016, CDC tried to culture the pox variants and while the attempts failed, they at least determined that the viral remnants presented no infection risk. The culture testing is an important public health measure that provided further evidence that scabs and other historical and ancient pox materials (while genetically viable) are not contagious. By the end of November, Dr. Hendrik Poinar and Dr. Ana Duggan from the McMaster Ancient DNA Centre at McMaster University, Canada, joined the conversation with CDC. Experts on ancient DNA extraction, the McMaster team recently identified a strain of cholera in a fluid-preserved specimen of cholera-infested tissue that has been preserved since 1849 at the Mutter Museum.[2] The cholera identification marked the first instance of viable DNA extraction from a 19th-century fluid-preserved specimen - an etiological milestone. Could the McMaster team do with the pox specimens what the CDC was not able to do? Could the McMaster laboratory repeat the breakthrough, but with Orthopoxvirus this time? If the Mutter's 19th-century specimens are not smallpox, then what are they? If CDC's suggestion that the scabs derive from an animal virus is true, then what animal? Nineteenth-century sources maintain that doctors experimented with

---

[1] Multiple instances of the inadvertent discovery of early vaccine material have occurred in North America in recent years. The CDC's assessment of these cases is detailed in Andrea M. McCollum *et al*., "Poxvirus viability and signatures in historical relics," *Emerging Infectious Diseases* (2014) 20(2):177-184.

[2] Devault, Alison M. *et al*., "Second-Pandemic Strain of *Vibrio cholerae* from the Philadelphia Cholera Outbreak of 1849." *New England Journal of Medicine* (2014); 370:334-340, January 23, 2014, DOI: 10.1056/NEJMoa1308663; Alison M. Devault, et al., "Ancient pathogen DNA in archaeological samples detected with a Microbial Detection Array." *Scientific Reports* (2014) **4**, Article number: 4245 doi:10.1038/srep04245

obtaining virus from many animals.[3] In addition, the Mutter had five vaccination kits with multiple components; CDC was only able to obtain three hits from scab and lymph material. Could the McMaster team do better?

In April 2017, the Mütter Museum received permission from the World Health Organization to transport the vaccination kits to the McMaster Ancient DNA Centre from CDC and continue the research. By early July, the McMaster team had been able to obtain low coverage genomic information from the scab material.[4] Perhaps more importantly for the work planned here, they were also able to obtain genomic level DNA from the lancet, an empty tin that had previously held scab material, and glass slides with no apparent indication of any tissue/sample. **These results show the technological means now exists to engage in a comprehensive historical vaccination study with and importantly *without* destruction of remaining scab or organic tissues**. From each of the five kits sampled, a viral genome of 19th century vaccinia was recovered and computationally reassembled. The high coverage complete genomes were generated from scabrous material, a substance believed to be lymph, and an empty tin box. Viral molecules were also recovered from the glass slides and lancets, though too few to generate more than partial genomes. The research generated from this Mütter/McMaster collaboration was published in July 2020 in the journal *Genome Biology*[5]

Evolutionary Significance

It is impossible to underestimate the global effect of smallpox and the triumph of modern medicine with its eradication through vaccination. Yet for a disease once so feared, and a vaccine so successful, we know remarkably little of the biology and evolutionary histories of the implicated viruses. It is clear that *variola* virus, the etiological agent of smallpox, is a human specific virus, yet when it began infecting humans or from what previous host it jumped remains unclear. Similarly, cowpox has typically been implicated in the development of Jenner's vaccine though Jenner himself also references horsepox. By the late 19th century, smallpox vaccines made use of *vaccinia* virus, but the provenance of that virus is unclear[5]. What is clear is that a variety of other Orthopoxviruses, including those capable of imparting immunity against *variola* virus, can infect a variety of other vertebrate hosts. A 2016 study from the McMaster team suggested a very recent ancestor for all *variola* virus strains circulating in the 20th century indicating that vaccination campaigns may have been imparting severe genetic bottlenecks on the diversity of virus strains worldwide[4]. The examination of historical samples, both of smallpox and vaccination sources, will allow us to clarify some of the outstanding questions regarding the

---

[3] Ramsey, Frank A., "Abnormalities of vaccination." *Researches upon 'Spurious Vaccination,' or the Abnormal Phenomena Accompanying and Following Vaccination in the Confederate Army during the Recent American Civil War, 1861-1865*. Joseph Jones, compiler and editor. Nashville: University Medical Press, 1867, 96

[4] Duggan, A.T., *et al*., "17th Century Variola Virus Reveals the Recent History of Smallpox." *Current Biology* (2016) **26** (24): 3407-3412

[5] Duggan, A.T., Klunk, J., *et al.* The origins and genomic diversity of American Civil War Era smallpox vaccine strains. *Genome Biol* **21,** 175 (2020). https://doi.org/10.1186/s13059-020-02079-z

evolutionary history of these viruses; the origin of *vaccinia* as a viral strain potentially produced through human intervention and recombination during early vaccination campaigns; the potential reservoir of human-specific *variola* virus; and the relationship between cowpox, horsepox and *vaccinia* virus.

Historical Questions

The evolutionary questions mirror historical ones. Until the production and management of controlled cattle bred for vaccine standardized its manufacture, the precise substances used for inoculation remain conjectural.[6] Apparent pox-related diseases could not be categorized genetically before the 20th century without analyzing historical disease remnants, so historians cannot easily distinguish between Orthopoxviruses and what were termed "eruptive skin diseases" in the historical literature. Clarissa R. Damaso (see note 7) has meticulously traced the evolution of Beaugency cattle for vaccine production beyond the 1870s, by which time the circulation of pox via human bodies was no longer necessary. Before that time, the genetic pox map is imperfectly known, compounding historical uncertainty. Early pox outbreaks influenced population dynamics—from mortality rates to migration and settlement—and played a strategic role in military campaigns. George Washington's adroit management of pox outbreaks in the Continental Army during the American Revolution, for example, contributed to battlefield victories that strengthened foreign confidence in the nascent nation and encouraged Dutch and French financial and military aid, respectively. How did 18th century pox, however, influence early United States-Native American relationships, and to what extent and with what effect did European empires exploit pox to gain Native American support against the fledgling United States? These questions indicate the range of historical problems that could be illuminated by the genetic history of pox and their contrasting vaccines produced of unknown viral strains.

Objectives, Outcomes, and Dissemination

Our group has established several main objectives:

1. Create a comprehensive database or information network that will aggregate these separate scientific and historical elements into a comprehensive map with linked data showing the dissemination (migration) of vaccination of smallpox throughout Europe and North America with the aim of eventually creating a global map.
    a. This body of work will attempt to answer the following questions that are vital both to scientific and historical understanding:
        (1) How did the vaccination/variolation strains move throughout the world?
        (2) What strains were used?
        (3) How did they change over time?
        (4) Were certain strains more successful than others?

[6] As a human-devised process to create biological material, "manufacture" is indeed the correct term.

2. Create a database of historical/ancient DNA samples of putative Orthopox (or other viral) specimens from museum, archives, and other institutions across the globe.
    a. The DNA can be obtained from biological samples through destructive analysis and non-destructive swabbing vaccination kits, rinsing scab containers, or taking samples of the preserving fluid of a biological specimen.

3. Create a body of archival and historical research material around the material culture of specimen samples by examining the provenance of the objects, such as the physicians who used the kits or donated the specimens, their era of activity, geographic location, their correspondence and publications, and their vaccination success rate, among other factors. The goal is to get DNA from these materials to inform the origin and evolution of the virus and from the host to see who was "carrying" the virus.

4. Share findings with historians and modern public health workers. Whenever possible, we plan to make databases open source. While the information is historical in nature, it has modern day significance in helping public health workers understand how viruses spread and how vaccination protocols were implemented in the 18th through early 20th centuries.

5. Publish findings in peer-reviewed journals. Members of our team have impressive publication credentials including *The New England Journal of Medicine*, *Nature, Science*, *Current Biology, and Genome Biology*.

Institutions Involved in the Project

1. **The Mütter Research Institute:** The research arm of the Mütter Museum of the College of Physicians of Philadelphia, the Mütter Research Institute (MRI), establishes collaborations and partnerships with other historic, scientific, or medical institutions with the goal of utilizing historical collections to conduct research relevant to 21st –century human health. The Institute is headed by Anna N. Dhody, Director of the MRI and Acting Co-Director and Curator of the Mütter Museum. For more information please see: http://muttermuseum.org/institute/

2. **McMaster Ancient DNA Centre**: Dr. Hendrik Poinar uses DNA recovered from a variety of archaeological, paleontological and forensic contexts to answer questions of evolutionary or biological interest. Dr. Ana Duggan is an expert in the *in silico* reconstruction of ancient viral genomes and the evolutionary history of variola virus. She led the research of both the 16th century Lithuanian smallpox strain[4] and the Mütter vaccination kits[5]. The McMaster aDNA Centre will process samples for extraction of genetic material, sequence the DNA, analyze the resulting data in an evolutionary perspective, and return any remaining material to the institution of origin. For more information see: http://socserv.mcmaster.ca/adna/

3. **Marie Bashir Institute for Infectious Diseases & Biosecurity, University of Sydney**: Prof. Edward Holmes is the world leader on the evolutionary analyses of both viral and bacterial pathogens. He will be instrumental in the analysis and interpretation of the results obtained from these kits.

4. **Centers for Disease Control and Prevention (CDC)**: Epidemiologist Andrea McCollum, PhD, analyzed the Mütter Museum objects and has investigated other examples of historic vaccine materials that were discovered in United States collections. As a member of the poxvirus team at CDC, she has led outbreak investigations in the Democratic Republic of the Congo (DRC) and conducted several investigations regarding exposures to poxviruses in the U.S. She currently works to enhance surveillance for monkeypox, improve health-care worker capacity, and improve laboratory diagnostics in the DRC. For further information, see: https://www.cdc.gov/csels/index.html

5. **Department of Pathology, University of Cambridge**: Professor Geoffrey L. Smith. Wellcome Trust Principle Research Fellow. Head of the Poxvirus Research Group. Dr. Smith is an expert in *vaccinia* virus and how it evades the host immune response by the expression of key proteins. His expertise will be crucial in the interpretation of the old vaccinia virus genomes we reconstruct from these ancient kits.

Collaborator Logistics

We invite your institution to join our project as a collaborator on this exciting large-scale project. We ask that you:

- Collaborate on determining samples in your collections that might be of interest.
- If interested provide access to said materials for sampling or rinsing.
- Provide access to any associated data about the material.
    - Data include catalog records, associated archives, donor information, correspondence, and published accounts.

If you join us as a content collaborator, we offer your institution:

- Co-authorship on any published papers using data from your object(s).
- Acknowledgement in the database as a contributor and collaborator.
- A member of our team would come and give a talk about the project to your institution.

Funding Needs

We are not seeking funding of any kind from your institution. The project has the necessary internal funding for this phase of the project and is actively seeking additional funding to expand.

Sample Needs

We seek your institution's collaboration to sample any smallpox-related vaccination material in your collection. We are interested in the following types of objects:

1. Vaccination Kits
    a. Either entire kits or any potential kit components where pox material may have come in contact with the object/instrument. Components include lancets,

glass plates, tin boxes, small glass vials, ivory or bone points, quills, capillary tubes, and other containers for tools or vaccine material.

b. Nineteenth century homeopathy medicines may contain pox material as vaccination was considered a homeopathic therapeutic practice. Vaccination tools or scabs may be found within homeopathic medical kits.

2. Scabs (called "crusts" in historic sources) or lymph material used in vaccination.

3. Samples thought to have been infected with smallpox, including archival documents and media, fluid preserved specimens, osteological specimens, tissue samples, microscope slides, or other materials.

Our experiences thus far have found these objects are often associated with a variety of different terms such as: smallpox, small pox, variola, kinepox, kine pox, kine pock, cowpox, horsepox, vaccinia, and vacciniae variolae.

Sampling Protocol

Most ancient DNA research involves destructive sampling; however, our initial results suggest that even in the absence of any (visible) biological materials we can obtain high-quality and plentiful genomic information. We therefore suggest non-destructive sampling of objects that may have come in contact with vaccination material. Destructive and non-destructive tests are distinguished as follows:

1. Non-destructive sampling: For inorganic materials such as glass slides, the sample is submerged or rinsed (necessary for tin vaccination boxes or blades) within a buffering solution. Following the contact with buffering solution, samples are carefully dried with a Kimwipe and then air-dried for at least an hour before resealing them. As the exact composition of these items may not be known, we cannot be certain how they will react with the buffer, however, objects from the Mütter Museum were processed as described above and to date have shown no signs of deterioration.

2. Destructive sampling (for organic material such as scabs, teeth or bone): Tiny portions of the sample are cut or drilled. Note that the McMaster aDNA Centre recovered the *vaccinia* strain from a 13mg subsection of one scab. To recover material from bone, a small piece is drilled, whereas for teeth, a section of the root below the cementoenamel junction is cut, leaving the crown of the tooth untouched. While every effort is made to destroy as little material as possible and to drill from portions that have already been damaged or are inconspicuous, samples can nevertheless shatter on contact with the drill.

3. Minimally destructive sampling: For fluid preserved samples, the McMaster team is considering a protocol wherein fluid would be collected from around the preserved specimen, along with any sediment or detached pieces of tissue from the bottom of the preservation jar. Please note that this protocol has not yet been tested to verify its potential for the recovery of DNA but, if successful, it should allow wet organic specimens to be sampled without damaging the tissue itself.

Vaccination kit manufactured by J. H. Gemrig, a major supplier of medical instruments to the U.S. Army during the Civil War. Credit: Accession number 17090.29, collection of the Mütter Museum of The College of Physicians of Philadelphia.

Method of Analysis

Following sampling, we will extract DNA, including DNA of the host, the virus, and any environmental DNA present in the sample (as sources of contamination), and prepare the molecules for sequencing on an Illumina platform (a widely used next-generation sequencing system). We may attempt to sequence all the DNA (so called "shotgun sequencing") or we may use a capture (or "targeted enrichment") approach to sequence molecules originating from Orthopoxviruses only.

Once we have generated the sequencing data, all remaining analysis will be strictly computational, and we will be able to return any material to their home institutions. From the sequencing data, we will attempt to identify any molecules that match known Orthopoxviruses, to reconstruct as many viral genomes as possible, reconstruct evolutionary history, and answer the questions posed under Objective #3.

Additional Research

In addition to our project, there are other researchers engaged in historic smallpox study. We are in dialogue with them in hope of a future collaboration. These researchers are:

> José Esparza, Ph.D., Institute of Human Virology, University of Maryland School of Medicine. Dr. Esparza is working with Andreas Nitsche, Ph.D., Robert Koch Institute in Berlin, and Clarissa Damaso, Ph.D., poxvirologist at the Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro.[7]

[7] Esparza, Jose, "Has horsepox become extinct?" *Veterinary Record* (2013 Sep 21) **173** (11):272-3; Clarissa R. Damaso, "Revisiting Jenner's mysteries, the role of Beaugency lymph in

We hope the other researchers will collaborate with us as we are working toward the same goal. Our team is committed to the spirit of shared knowledge.

Conclusion

We hope that your organization will consider collaborating with us on this exciting project. In order to succeed in our goals we need your participation to provide materials for testing. The promise of this project is substantial: tracking the path of vaccination will yield data of immediate epidemiological value. The project will also illuminate the historical record. For the first time, we may identify the substances used to vaccinate people before the 20th century and understand historical vaccination successes and failures. Our ability to combat emerging diseases rests in our understanding of how they evolve, mutate, and affect humans, and this project will provide unique insights into these key questions. This convergence of scientific discovery and historical investigation promises very exciting results.

Primary Contact

Anna N. Dhody, MFS
Executive Director
DhodyResearch Institute
andhody@gmail.com
December, 2023

---

the evolutionary path of ancient smallpox vaccines." *The Lancet*, August 18, 2017 (published online), http://dx.doi.org/10.1016/S1473-3099(17)30445-0.