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SMALLPOX WATCH

Frozen mummies and envelopes of scabs could contain remnants of one of history's most prolific killers.

In 2011, while construction workers were digging a foundation at a site in Queens, New York, their equipment struck against something metal. Then a body rolled out of the rubble. Thinking that they might have unearthed the shallow grave of a murder victim, the workers immediately called the New York chief medical examiner's office, and forensic anthropologist Scott Warnasch drove over with a team to check it out.

The body, which had probably been buried in the cemetery of a nearby church, turned out to be a mid-19th-century mummy — of an African-American woman dressed in a nightshirt and socks who had been exceptionally well preserved by her ornate iron coffin. The find struck the forensics team as odd: a black woman in the mid-1800s was unlikely to have been able to afford such a luxurious resting place.

Then the examiners noticed the lesions and raised bumps that covered the corpse. The marks reminded Bradley Adams, New York

BY SARA REARDON

City's chief of forensic anthropology, of photos he had seen of smallpox victims. The pricey coffin with its airtight seal, the scientists realized, might have been meant not to preserve the body of a wealthy individual but to quarantine an infection. "We took a step back," says Warnasch. The site instantly changed from potential crime scene to potential biohazard, and the city's public-health department called the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, for advice.

CDC officials reassured the New York investigators that the risk of infection was low. But the agency quickly dispatched epidemiologist Andrea McCollum and a team of scientists in protective clothing to autopsy the body and retrieve tissue samples. No one knows how long the smallpox virus can survive in a human corpse, and McCollum's team hoped to recover DNA or even viable virus particles from the woman's body.

This month, the World Health Assembly —

the decision-making body of the World Health Organization (WHO) — will meet in Geneva, Switzerland, and decide when to destroy the only known stocks of smallpox virus, held in deep freezes at highly protected laboratories in the United States and Russia. It is a move that has been delayed since the 1980s, and in all likelihood will be put off yet again. But even if the official stocks of virus are destroyed, the chance remains that other batches of the virus could be hidden in a freezer somewhere — or that the pathogen could re-emerge, zombie-like, from a mummified corpse such as the dead woman found in Queens.

Although the risk of such a virus causing a pandemic is low, says McCollum, "it's a very real concern". And an opportunity. In fact, she and a handful of smallpox specialists rather hope that ancient poxviruses, dead or alive, are still out there somewhere. Researchers jump on tips that could turn up specimens reaching back decades or more, and then scour the remains for clues. Practically, such a find could reveal just how long the smallpox virus, known as variola, can



1. A frozen 300-year-old mummy, from a group found in an ornate coffin in Siberia, yielded DNA from the smallpox virus.
2. Forensic anthropologist Scott Warnasch examines a 160-year-old corpse that was accidentally unearthed in Queens, New York.
3. An 1876 letter containing a scab intended for vaccination turned up in a Virginia museum, setting off a minor scare.
4. Tests of the mailed scab showed that it contained DNA from a virus related to smallpox, but it was harmless.

survive under different conditions. And even if samples do not yield viable virus, researchers could potentially recover DNA from past infections that will add to our picture of how the scourge has evolved, perhaps offering insight into how we might mount defences should smallpox ever re-emerge.

TRACES FROM HISTORY

Smallpox has a reputation as one of the worst diseases in history: it spreads quickly through the human population and kills about one-third of those infected. Although it has afflicted people throughout the world, Native Americans and Africans suffered in extreme ways: some populations all but vanished after contracting the disease from European settlers between the sixteenth and eighteenth centuries. In 1966, when an estimated 10 million to 15 million cases were still occurring worldwide per year, the WHO decided to step up its strategic vaccination and containment campaigns and, by the end of 1977, had eradicated the disease.

Yet remnants of variola remain scattered around the world. Signs of the disease, including skin lesions, virus particles and smallpox DNA, have turned up in stored human scabs and corpses as old as the 3,200-year-old mummy of Rameses V (ref. 1). But no one has ever reported the recovery of live virus from a dead body.

To the CDC's disappointment, the woman from Queens was no exception: the smallpox DNA in her corpse had degraded, leaving no trace of the virus. In theory, other mummies

could harbour the virus, although the risk of infection is slight, says Peter Jahrling, a virologist at the US National Institute of Allergy and Infectious Disease in Frederick, Maryland. "If you dig up one of these things, you might want to take some precautions," he says. Cold climates, in particular, may preserve the virus better. "I think it's plausible to imagine that virus might exist in mummies in cold crypts," says Jahrling.

"No one knows how long the virus can survive in a human corpse."

The virus is extraordinarily stable in human tissue, notes D. A. Henderson at the University of Pittsburgh Center for Health Security in Baltimore, Maryland, who led the WHO's smallpox eradication efforts in the 1960s and 1970s. When the characteristic bumps on a person's skin burst, blood coagulates at the site and fibrous proteins weave a mesh around the virus, trapping it inside a scab. Even after the scabs flake off, the caged virus is unlikely to become airborne or to stick to a person's skin if touched. This stability allowed physicians to develop an early form of immunization known as variolation, in which a small bit of a smallpox scab was inserted into a cut in the skin — causing, for reasons that remain unclear, a milder case of smallpox than a normal infection would have done.

Variolation was used into the twentieth century, especially in poorer communities, although in the nineteenth century many physicians switched to vaccinating with the related, but much less harmful, vaccinia virus. With both viruses, scabs were a useful tool: doctors carried them around in their cases and the scabs could even be mailed, as the Virginia Historical Society (VHS) Museum in Richmond discovered in 2010 when it came across an unusual envelope in its collection. In a letter dated 1876, William Massie, who had recently moved to Richmond, Virginia, wrote to his father Henry in Charlottesville. "[I] hope that this will reach you in faculty of time," he had scrawled in spidery handwriting. Pinned to the letter "so that you cannot lose it as you did before" was a twist of metal foil containing a thumbnail-sized scab that was "perfectly fresh and was taken from an infant's arm yesterday". It should be enough to vaccinate 12 people, the younger Massie wrote.

VHS president Paul Levensgood thinks that the artefact is an enchanting glimpse into history. "It's one little story of one family trying to protect itself from something that was this horrifying bane of human existence," he says. "Charlottesville would have been the hinterlands in 1870s; maybe you had access to the vaccine in the big city, but your family wouldn't, so here you were trying to keep them safe."

The museum displayed it along with its letter in an exhibit of "oddities". A few months later, Levensgood received a call from the CDC. Someone had alerted the agency after



The skin of Rameses V is covered with bumps, suggesting that he died of smallpox.

seeing a review of the exhibit in the newspaper.

McCollum and a colleague drove up to collect the scab, hopeful that this one would contain viral DNA. Molecular virologist Inger Damon, who heads poxvirus research at the CDC, says that the oldest known viable sample of variola is from 1939. “To get a sample that came from the 1800s would be enormously important,” she says. Studying the virus’s evolution could reveal when its ancestor emerged from an animal, or whether poxviruses have evolutionary tricks for stepping up potency, which may be relevant when studying viruses such as monkeypox, which is of increasing concern in Africa.

The museum’s scab did contain degraded viral DNA, yet it probably belongs not to variola but to a closely related poxvirus. The CDC group is still waiting for the results of the complete 186,000-base-pair genome sequence “I think they were genuinely disappointed,” Levengood says. Just to be safe, the researchers irradiated the scab before returning it to the museum, where it is now kept in a plastic lab tube — albeit a crumble of its former self because of the radiation.

The researchers at the CDC still hope that they will come across a good DNA sample from such a source. “We have little snippets of information from vaccine scabs that fell out of books or were squirreled away in attics,” says Damon. One such cache was stashed inside an envelope in an old book in a New Mexico library, and fell out when a librarian opened the volume in 2003.

Stored scabs have provided the best evidence for how long the virus can live. In an study begun in the 1950s, researchers in the Netherlands collected scabs freshly shed from people with smallpox and kept them in envelopes. They were able to isolate variola virus for 13 years, when they ran out of scabs, and the experiment has not been repeated. Nevertheless, the findings do show that the virus can survive for more

than a decade in a temperate climate².

A more likely source of infectious virus would be frozen bodies. Influenza viruses seem to be able to survive freezing in lakes and may thereby infect migrating birds³. And in February 2014, researchers studying Siberian permafrost reported that they were able to resurrect a giant 30,000-year-old virus that infects amoebae⁴. As global warming continues to melt the permafrost, it is possible that viral diseases could be released from the ice, the authors say.

SIBERIAN MUMMIES

Human activities such as drilling, construction and archaeological digs too, could unearth a viral cache. In 2004, a team of anthropologists hunting for mummies in Yakutia in Eastern Siberia turned up a gravesite containing five well-preserved frozen mummies thrown together in an ornate wooden coffin. The group — probably a family — seemed to have died suddenly in the early 1700s.

The best-preserved body, that of a young woman, had traces of variola DNA in her tissue. The DNA was too degraded for the scientists to reconstruct its entire sequence, but there was enough to show that it was a separate strain from those that circulated widely in Europe and Asia during the twentieth century. The scraps of DNA allowed the researchers to expand our knowledge of the evolutionary history of the smallpox virus, and such studies could eventually help to show how it spread around the world⁵.

Scientists at Russia’s VECTOR lab in Koltsovo had been thinking about this possibility years earlier. In 1991, a team set out for another village in Yakutia to try to isolate virus DNA from a handful of corpses that had been unearthed by a flood. The researchers were unable to recover any viable virus, or even any dead virus that had retained its shape. “It was a great disappointment,” Henderson says. The

tissue was well preserved and the virus should have been present. But the Russian scientists were among the best in the business, he says. “If they couldn’t get it, it was ungettable.”

The Russian researchers say that they have not repeated the expedition. And such hunts for live virus remain a low priority for funding organizations such as the WHO. Of more immediate concern is the ease with which someone could synthesize the virus in a clandestine lab: variola’s genome sequence has been available since the 1990s⁶. WHO assistant director-general for health security Keiji Fukuda says that member states will discuss that possibility at the World Health Assembly meeting this month and put together a working group to assess the extent of the threat.

Another concern is that smallpox could escape from a secret cache. Few biosecurity specialists believe that the two stocks kept at the CDC and VECTOR are the only ones in existence. For instance, variola could very well be in the freezer of someone who defected from the Soviet Union, says Jahrling. Perhaps even more worrying is that someone could fairly easily tinker with an existing poxvirus to change its host species, make it more resistant to drugs or coax it to spread more easily.

All those possibilities — along with the chance that ancient smallpox could resurface — has led some researchers to argue that stocks of the virus should stay available. Most people born since the virus was eradicated are not vaccinated against the disease, partly because there are risks associated with the vaccine. And keeping smallpox around to allow the testing of new treatments for the disease makes sense, Jahrling argues. His group has been developing antiviral drugs to treat a smallpox infection after it occurs. “I’m impassioned to keep research going for a while,” he says.

Damon adds that newer, safer vaccines are being developed and that having the original virus around could help with testing these as well. She hopes that the decision to destroy US and Russian stocks will once more be delayed. “I don’t think we will ever know everything we could possibly know.”

In the meantime, researchers continue to piece together the snippets of history they can glean from the occasional corpse or scab. “It’s one of these great examples,” Levengood says, “of a tiny object that can open up this enormous story.” ■

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