Golden Silkworms in Pandora's Box:
the threat posed by viral gain-of-function research

In ancient China there was a tradition of developing a special poison called *Gu* by throwing as many venomous creatures as you could find into a jar and sealing them in, letting them kill each other until only one was left. Also known as a "golden silkworm," the lone surviving creature was then thought to host a "demonic poison" since every other creature's venom was thought to concentrate within it. According to Chinese folklore, this golden-silkworm could then mutate itself into any number of other animals – retaining its lethal ability no matter what form it took.

Turns out that manipulating nature in an attempt to create unpredictable and unnaturally powerful weapons is nothing new. However, this ancient practice took a modern turn about 50 years ago, creating a threat to humanity that may have just reemerged from its container once more.

Back in 1977, a very peculiar epidemic began to sweep across Russia. Once scientists had isolated it, they discovered it was a rather unique strain of the H1N1 Swine Flu, a variant of the virus that'd caused the 1918 "Spanish Flu" pandemic. However, this particular variant of the H1N1 Swine Flu had something quite distinctive about its genome. At the time, the Soviet Union was employing tens of thousands of scientists designing every possible flavor of biological weapon, a rabidly immoral weapons program with a spotty safety record – pathogens were known to leak out of Soviet labs almost regularly. And Soviet scientists were reported to bring dead research animals home for dinner, meat wasn't exactly readily available in the USSR at that time, which parallels the reports of scientists in Wuhan smuggling dead lab animals out to sell for a few extra bucks on the street. And as far as lab leaks go, China's labs have leaked the SARS virus four times just in recent years. Even more specifically, a delegation from the State Department visited Wuhan's Institute of Virology in early 2018 and asked for more resources for the lab since "the new lab has a serious shortage of appropriately trained technicians and investigators needed to safely operate this high-containment laboratory," going on to emphasis how grave the consequences would be if a lethal virus managed to escape that lab.

Lab leaks are nothing new for high-level virology labs across the world, and provide an avenue for COVID-19's entrance onto the world stage that is just as viable as a natural zoonotic transmission, which you'll soon learn doesn't really fit the facts either. And leaks tend not to happen entirely randomly, the odds they occur are roughly paired with the pace of research into specific types of viruses.

For example, earlier in the 70's before the Soviet H1N1 Swine Flu leak, "the swine flu scare… [had] prompted the international community to reexamine their stocks of the latest previously circulating H1N1 strains to attempt to develop a vaccine," which was seen to have increased the odds that someone, somewhere would make a mistake and leak an altered strain of the virus out of their lab. This increased pace of research mirrors recent times, when scientists have been

investigating and trying to understand the supposedly impending threat posed by coronaviruses for years, capturing as many unique strains from the wild as they could, and mixing and matching their genomes in the lab.

In the years that followed the 1977 leak, genetic analysis looking to determine where this particular strain of H1N1 Swine Flu came from found something rather odd: It was very similar to strains of H1N1 that hadn't been in circulation for decades, and seemed to be the product of "sequential passage in an animal reservoir," which was determined by its vast genetic distance from any other existing strain of flu. Curiously, this was also what was found with COVID-19, which also appears so distant from any related coronavirus that it's been placed in its own clade, an isolated branch way out on its own in the viral family tree – meaning it's the lone example of its kind, and doesn't clump together with all the other known coronaviruses.

An increased pace of research into the H1N1 Swine Flu back in the 1970s increased the odds that a mistake would happen until one eventually did, and a leak occurred. So maybe it's worth keeping in mind that our current pandemic was preceded by years of research into coronaviruses everywhere from the University of North Carolina to the Wuhan Institute of Virology's Disease Engineering Technical Research Center, and have been accelerated a massive push by the Chinese military to expand their biotechnological capabilities as well specific events like by a massive international conference meant to study a potential pandemic caused by a hyper-virulent strain of coronavirus, Johns Hopkins' Event 201. This international conference was funded primarily by the World Economic Forum as well as the Bill and Melinda Gates Foundation, and notably occurred in October 2019, just weeks before the start of COVID-19's outbreak.

Leading up to 1977 an increased pace of research into strains of the flu was seen to increase the odds that an accidental leak would occur, so shouldn't the same logic should be applied to the start of our pandemic today? Why is almost everyone today assuming that the increased pace of research means scientists *anticipated* this outbreak, instead of *causing* it? Wouldn't an increased pace of research also increase the odds that a leak of a lab-modified coronavirus would occur, just like an increased pace of research precipitated the leak of the engineered H1N1 Swine Flu back in the 1970s?

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Scientists have been directly altering and modifying viral genomes for at least the past twenty years, doing everything from building complete viruses from scratch, to tweaking them and then passing them through series of animal hosts to artificially speed selection and evolution along so that they're able have as many different strains of virus with as many novel features as possible to manipulate. There's nothing inherently sinister about this research, mastering the manipulation of viral genomes has given scientists invaluable insights about how viruses evolve, replicate, spread, and kill – allowing novel methods of to fight back against them to emerge as our understanding of them has grown.

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And so most of this work didn't really raise too many eyebrows, until about ten years ago when scientists in Stony Brook, NY – not coincidentally also the first place to build a DNA-virus from scratch – took the H5N1 Bird Flu, tweaked its genome in two places, and then passed it through a series of ferret hosts in the lab until it became airborne. This sort of research, a minor alteration and then serial passage through ferrets, did two things: Resulted in a virus that would *look* natural and wouldn't *appear* to have been directly genetically altered, and also created a virus that was way out on its own branch of the viral family tree since those sequential passages added generations far faster than they'd naturally occur in the wild. If that sounds familiar, maybe that's because those traits are also exactly what's found with COVID-19.

Quite curiously, one of the scientists supporting gain-of-function research in an article that noted the virus "could change history if it was ever set free" appeared on Joe Rogan's podcast in 2020 a few weeks into the current pandemic, claiming that COVID-19 was definitely natural and making no mention of this animal-based dual-use gain-of-function research at all. Odd, right? It's almost like Michael Osterholm, whose entire career rests on advancing gain-of-function of research, might want to whitewash what's really going on.

Osterholm failed to tell the story of this genetically modified H5N1 Bird Flu, which was turned into a virus that "could make the deadly 1918 pandemic look like a pesky cold." This result was so troubling that the NIH, which had funded the research, tried to make sure that the it would only be published after enough details were taken out to make replication of the experiment tough to perform. However, one of the virologists involved in the research thought these restrictions were a bit silly, since the gist of the experiment was enough to allow anyone with enough money to replicate them without a problem. Especially researchers who were already familiar with manipulating bat coronaviruses, two of whom learned how to do exactly that at UNC in 2015 before returning to Wuhan to continue their work.

If you still aren't sure whether the scientists involved with kind of research are being forthright, there's Dr. Ralph Baric. It was in his lab at UNC that a hyper-virulent bat Franken-virus was created by splicing a new protein-spike on an existing coronavirus, creating a monster so vicious that a virologist with the Louis Pasteur Institute of Paris warned: *"If the [new] virus escaped, nobody could predict the trajectory."* It should also be noted that several years prior to tinkering directly with bat coronavirus spike-proteins, Baric orchestrated research that involved isolating a coronavirus from civets and then passing it through mammalian ACE2 receptor cells that were grown in the lab from kidney and brain samples – gain-of-function serial passage through host cell lines instead of entire hosts which imparted a strong affinity for ACE2, and presumably created another airborne strain of coronavirus. And if cells derived from kidneys and brains were used for the serial passage development of COVID-19, as they typically are for this purpose, that might help explain its affinity for attacking the kidneys and brains of its human hosts.

More recently, corporate executives trying to pass as scientists like Peter Daszak and his company, EcoHealth Alliance, which is a non-profit that depends largely on multi-million dollar government grants to function, have been partnering with Chinese researchers in an attempt to secure funding for more and more research into coronaviruses. In one of the more transparent

attempts at PR-spin, Daszak was featured alongside one of the researchers who learned how to create hyper-virulent bat coronaviruses in Baric's lab at UNC during the aforementioned research back in 2015, Zhengli Shi. This article insists we should take Zhengli at her word when she claims to have not found a match after she checked COVID-19's genome against everything in her lab. As if someone responsible for releasing the most virulent pathogen to hit humanity in modern history, one that's already killed thousands and is projected to kill millions and millions more all across the globe, would simply fess-up to it, torpedoing her career and the years of research performed by her and her colleagues. No even halfway rational person would open themselves to the legal repercussions that would inevitably follow such an admission.

And if Baric was being honest, you might expect him to warn the public about the lethal potential coronaviruses pose during our current outbreak, especially since in late January there was absolutely no way at all to know whether a lab leak was involved or not. However, when he was asked if the public should be worried about COVID-19 he said that people should be more worried about the seasonal flu. Pretty bizarre statement from a scientist who knew full well how dangerous coronaviruses could be – in this case COVID-19 is somewhere north of thirty-times more lethal than the seasonal flu – and given the fact that not only was Zhengli Shi working in his lab on that project in 2015, but Xing-Yi Ge was too. Both of whom returned to Wuhan where they've continued their virology work for years.

Xing-Yi Ge is especially notable since in 2013 he became the very first scientist to isolate a bat coronavirus from nature that uses the ACE2 receptor, which is found prolifically in human, tree shrew, and ferret lungs and allows coronaviruses to become airborne. And as you might have learned by now, that's the exact receptor used by COVID-19 to enter human cells – if anyone would know how to finagle that part of the coronavirus genome, it'd be him. So both Xing-Yi Ge and Zhengli Shi were part of the research team that created this hybridized hyper-virulent bat coronavirus under Baric, who's actively downplayed the risk posed by COVID-19, and then returned to work in Wuhan, where funding provided in part by Daszak's company allowed them to continue their work on coronaviruses with plenty of research to cut-and-paste into their work at the Wuhan Institute of Virology's Disease Engineering Technical Research Center.

As Dr. Ian Malcolm puts it in Jurassic Park, it is never a good idea to futz around with science and research when you don't fully understand it, nor its possible implications.

A few years later the NIH would ban this dual-use "gain-of-function" research, a ban that would remain in place from 2014 until 2017, when it was lifted. And what was the reasoning behind lifting the ban? To allow for research on flu viruses, as well as SARS and MERS – coronaviruses just like our new friend, COVID-19. Since then, hundreds of millions of dollars of funding poured into research on these viruses, supposedly with oversight meant to reduce "the potential to create, transfer, or use an enhanced potential pandemic pathogen."

Turns out, that oversight might not have worked out too well, witnessed by the thousands who have already died from COVID-19 and the hundreds of thousands more who are projected to die in the coming months. Despite this carnage, the scientific community at large seems to be

determined to pretend like this novel coronavirus almost certainly came from nature, when in fact the preponderance of evidence leans the other direction. As the most prominent example, the article "The Proximal Origin of COVID-19," published March 17, 2020 in *Nature Medicine*, concludes based on detailed sequence analysis that the origin of the novel coronavirus causing the current pandemic is "not a laboratory construct nor a purposefully manipulated virus," but is more likely the result of natural selection and a zoonotic jump. However, the assigned likelihood of a natural origin for COVID-19 does not logically follow from the very evidence the authors introduce and cite.

If COVID-19 has a novel optimal solution for its spike-protein key to bind to the ACE2 receptor lock, and if, as the authors suggest, this might have occurred by serial mutational events either in an intermediate host or after a zoonotic jump in humans, then logically it could also have occurred by selection of a virus after serial passage through laboratory cultures or laboratory animals, just like the 1977 H1N1 Swine Flu and the H5N1 Bird Flu. The only origin for the COVID-19 spike-protein that the sequence data excludes is deliberate introduction of the SARS spike-protein sequence to create COVID-19. Otherwise, there are no structural or functional data to distinguish among natural and engineered possibilities other than COVID-19's genetic distance from other coronaviruses, distance shared by the H1N1 Swine Flu that leaked out of a Soviet lab, which also points to a laboratory origin here.

Their paper leaves out the implications of the dual-use gain-of-function research tool of serial passage through animal hosts entirely, despite the fact that it provides possible explanations for every unusual characteristic of COVID-19's genome.

And it's not like this is some esoteric research technique, the artificial manipulation of the H5N1 Bird Flu was contentious enough to cause the scientists working on it to "brace for a media storm," which occurred after researchers at two different institutions tweaked its genome in a few places and then passed it through a series of ferret hosts until it became both airborne and incredibly virulent, and left behind a genome that appeared to be the result of natural, albeit accelerated, selection. That process of sequential passage through animal hosts leaves a genome that appears "natural" and not purposeful, since it does not leave a genomic smoking gun, and would appear to be the result of natural selection so long as its relationship to sister species is ignored. However, the artificial generations caused by forced serial passage through ferret hosts creates evolutionary distance, which is exactly what is found with the 1977 H1N1 Swine Flu and COVID-19, which is so distant from any relatives that it has been given its own clade.

Further occluding the natural appearance of COVID-19, a recent study that examines the neutral sites that are assumed to best show heritage found that pangolins are "very unlikely" to have served as a host at all. However, "for a precursor virus to acquire both the polybasic cleavage site and mutations in the spike protein suitable for binding to human ACE2, an animal host would probably have to have a high population density." The ferret ACE2 receptor is equivalent to the one found in humans, and they would certainly have a high population density if they were collected in a lab for serial passage gain-of-function research.

Serial passage also explains one of COVID-19's most distinctive feature: its polybasic cleavage site. Rather than emerging through the natural mixing of unidentified viruses as some have

proposed, "the acquisition of polybasic cleavage sites... has also been observed after repeated passage in cell culture or through animals." This is precisely the process understood to have led to the emergence of the H1N1 Swine Flu in the 1970s, as well as the aforementioned airborne and highly virulent gain-of-function enchanced H5N1 Bird Flu a decade ago, and remains a viable way for COVID-19 to have gained its own polybasic cleavage site. And in very specific wording, other researchers have observed that this cleavage site may "provide a gain-of-function to the 2019-nCoV for efficient spreading in the human population compared to other lineage b betacoronaviruses."

Although COVID-19 clearly wasn't built nucleotide-by-nucleotide as the perfect bespoke bio-weapon, that doesn't mean human engineering hasn't influenced its development using other genetic tools. For instance, its "efficient solution" for ACE2 binding that's been described as something that wouldn't be intentionally engineered, is exactly the result that could be selected for after serial passage through ferrets in lab. And studies examining COVID-19's infectivity in ferrets found that it spreads readily among them, and also appears airborne in that animal model, lending support to the idea that ferrets were used for serial passage.

Further support for possibility that serial passage through lab animals played a role in the creation of COVID-19 comes from an April 2020 pre-print, which found that coronaviruses that target the ACE2 receptor bind with ferrets cells more tightly than any other species except the tree shrew, which only scored about 2% higher. Tree shrews have also been used for serial viral passage, and were promoted in a 2018 paper out of China as a preferable host for laboratory serial passage since they're cheaper, smaller, easier to handle, and closer to humans evolutionarily and physiologically than ferrets. Pangolins however, formed a much weaker bond than either, and were clustered way down on the list along with a handful of other much more unlikely intermediate animal hosts.

As far as determining how COVID-19 made the jump into humans goes, none of the data points to a natural zoonotic jump occurring. Addressing the possibility of long-standing circulation of bat-derived coronaviruses in humans, which would indicate that a natural jump had happened since a long history of pervasive infections would mean the virus would've had enough time to adapt to human biology and learn how to exploit it, the authors cite a study published March 2018 that examined people who live in villages barely a kilometer away from bat caves. But that study revealed the opposite of what you'd expect in the case of a natural zoonotic jump: only two-point-seven percent (2.7%) of those villagers had antibodies indicating any past exposure to bat coronaviruses. It also sampled people living in Wuhan and found no evidence whatsoever of exposure to "SARS-CoV-like coronaviruses." So there is very little evidence of any exposure to these coronaviruses even in Chinese villagers living in close proximity to bat caves, and at the epicenter of the current outbreak, the city of Wuhan – no exposure was found at all. These data do not support that COVID-19 was circulating in humans anywhere prior to the outbreak began in Wuhan in the winter of 2019, and decreases the odds that a natural zoonotic jump occurred.

It seems misguided and dangerous to ignore significant portions of the history of science, especially when that history is some of science's most troubled. It was the accidental leak of the H1N1 Swine Flu in the 1970s that first began the discussion about the moral and physical hazards involved with dual-use gain-of-function research. And the creation of the extraordinarily

virulent H5N1 Bird Flu, using the same technique of serial passage through an animal host in a lab, that helped lead to the NIH imposing a moratorium on dual-use gain-of-function research from 2014 until 2017, when it was relaxed explicitly to allow influenza as well as coronaviruses to be studied. Not coincidentally, over the years the NIH has provided $3.7 of funding to the Wuhan Institute of Virology for its research into coronavirus propagation, and in return has almost certainly had access to the discoveries it's funded.

Until a natural origin of COVID-19 is fully established, the prospect that it may have been the result of this research practice must be considered, especially since serial passage through animal or cellular hosts explains every single element of its unusual genome and presentation – and when the implications of an unnatural origin are so extraordinarily profound.

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