Researchers pursue a vaccine to target an Achilles' heel of the coronavirus

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Allison Vilander, a member of the research team, works to isolate DNA for use in vaccine construction. Credit: John Eisele

Propelled by the COVID-19 pandemic, a Colorado State University research team is pursuing a vaccine that would thwart the novel

coronavirus using a genetically modified form of the well-known probiotic Lactobacillus acidophilus, a bacterium commonly ingested in yogurt and other foods for gut health.

The beneficial bacterium thrives exactly where the new coronavirus, SARS-CoV-2, drives its spike to invade the body: the mucous membrane. It is here—especially in tissues of the nose, mouth, throat, and respiratory system—that the pathogen gains entry and breaks into host cells, prompting cellular changes that trigger disease.

The CSU team has discovered that an engineered form of L. acidophilus holds promise in orchestrating immunity and preventing the ominous fusion of virus and host cell at two key sites on the invader's spike protein. These points amount to an Achilles' heel—a viral vulnerability that could be exploited to avoid the deadly respiratory disease COVID-19.

Coronavirus spike proteins, which initiate infection, project from the outer layer of each spherical pathogen, giving the virus its characteristic barbed crown, or corona.

"Lactobacillus acidophilus is an organism that has the capability to modulate the human immune response, so we're trying to take advantage of that with a vaccine strategy right there at the mucosal surfaces," Gregg Dean, lead investigator, said. He is a professor and head of CSU's Department of Microbiology, Immunology, and Pathology. "We're selecting two specific sites on the spike protein with critical function that really represent an Achilles' heel for the virus. If we can block that function, the virus can no longer bind to a target host cell."

Dean's focus on COVID-19 began like this: He recently received a $3.5 million grant from the National Institutes of Health to work on a vaccine against human rotavirus, a family of pathogens distinct from

coronaviruses that also attack the mucous membrane. Meantime, he has pursued a vaccine against feline coronavirus in research supported by the Morris Animal Foundation. When the COVID-19 pandemic flared, Dean's research team immediately pivoted. The scientists have combined related expertise to thwart coronavirus infection in people using a vaccine method that could be applied in multiple ways; he has requested additional funding from NIH.

"It shows how important it is to have these research projects in progress so we can hit the ground running against emergent viruses," said Allison Vilander, a member of the research team and an assistant professor in CSU's Department of Microbiology, Immunology, and Pathology.

The team also includes Tony Schountz, an associate professor and expert in zoonotic viruses, those transmitted from animals to people; it's a category that includes SARS-CoV-2 and other deadly coronaviruses. Collaborators at North Carolina State University also are contributing

Given Dean's simultaneous study of coronaviruses in people and cats, it might not be surprising that he's a veterinarian, a credential held by a number of biomedical researchers at Colorado State. The applicability of animal medicine to human medicine is well-known among veterinarians and is one key source of CSU's expertise in infectious disease. That expertise dates to the school's early days, when the founder of Colorado State's veterinary program zeroed in on tuberculosis passed from dairy cattle to people through unpasteurized milk and butter.

As science has progressed, the ties between human and animal medicine have grown clearer and are advanced by the One Health movement. Consider: Some of the world's pathogens—coronaviruses among them—jump from animals to people, meaning scientists must understand the role of animals in that transmission, starting at the molecular level. In addition, all medical technologies designed for people

must first be proven in animal models to gain federal approval. And innovations to combat illness—diagnostic tools, treatments, and preventive measures—often are similar or identical in humans and animals.

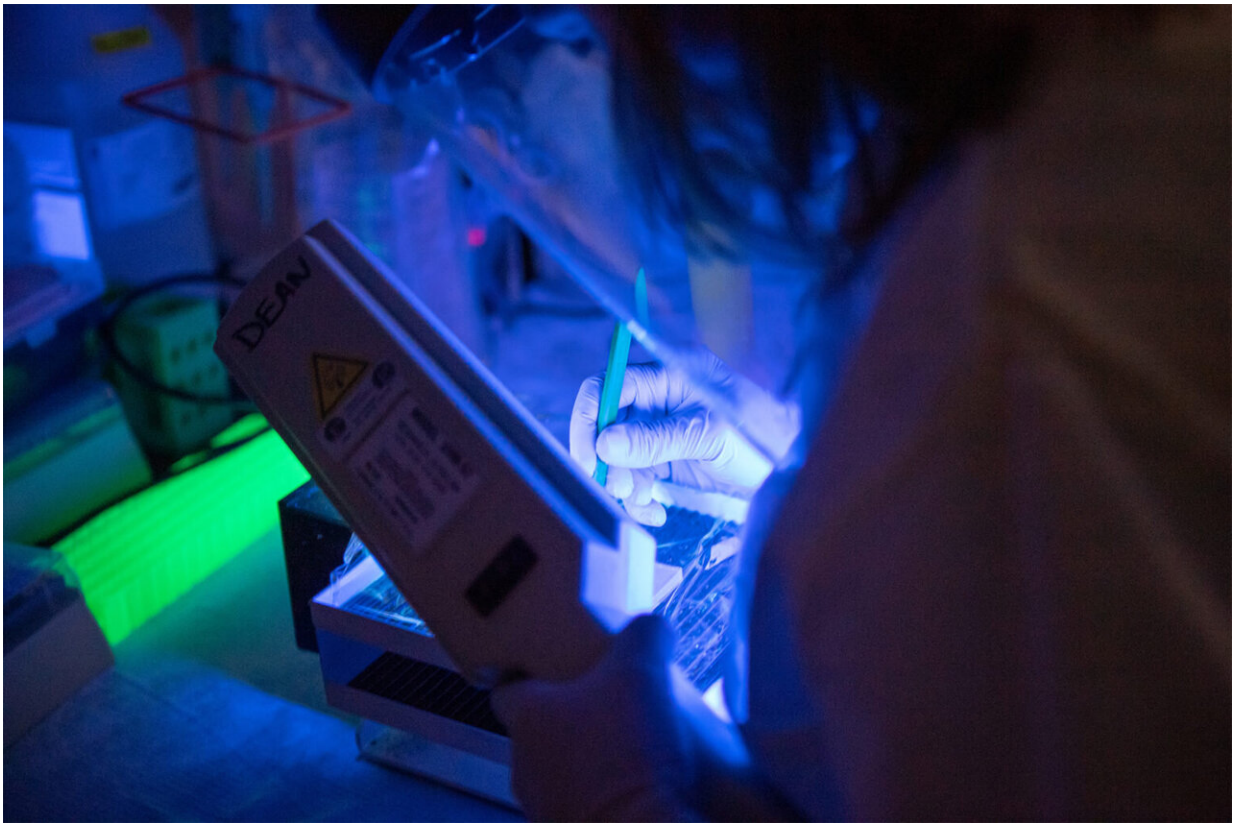
At CSU, home to the No. 3 veterinary school in the nation, biomedical expertise runs deep in animal and human health. "I don't know of another institution with this kind of portfolio," said Sue VandeWoude, an eminent veterinary researcher and director of CSU's One Health Initiative.

For Dean, the COVID-19 outbreak presents urgent research challenges whose solutions potentially could apply in two critical spheres.

"Coronaviruses are ubiquitous. Many species acquire them, so they've been a veterinary concern for decades," Dean noted. "The feline coronavirus is quite similar to the COVID-19 virus currently affecting humans in how it enters into a population and how you have this range of illness from nothing to very devastating. There have been numerous attempts to develop a vaccine against feline coronavirus, and they have not worked. But during the course of that research, we've learned a lot, and that has led us to this strategy for a human vaccine."

Another CSU team, led by Ray Goodrich, executive director of the University's Infectious Disease Research Center, is investigating a separate vaccine strategy—using ultraviolet light and riboflavin, better known as vitamin B2, to inactivate the new coronavirus. Goodrich has successfully used the photochemical innovation to reduce pathogens and prevent disease transmission through blood and blood products used for transfusions. When applied to a vaccine, the technology would achieve what Goodrich described as a "sterilizing immunity" against the invading coronavirus.

Both vaccine projects are part of a full-throttle scientific push, playing out in laboratories around the world, to develop the first successful and fully approved vaccine against COVID-19. Two vaccine candidates, one with NIH backing, entered human experimental trials on March 16. Dozens more are in the pipeline for assessment of safety and efficacy, the journal *Science* reported this week. Still, the process of bringing a vaccine to market will likely take 18 months because of extensive testing and required clinical trials.



Working with ultraviolet light, Vilander removes DNA bands from agarose gel following electrophoresis. The steps are a move toward constructing an experimental vaccine against coronavirus. Credit: John Eisele

"Every vaccine idea is a moonshot," said Dean, who has worked for decades on vaccines against human immunodeficiency virus, for which there still is no vaccine. "The vast majority of vaccine investigations are going to fail, so it's important that we have lots of scientists working on these urgent health problems. Even when vaccines don't reach the market, we are generating new knowledge that may be applied to other emerging infectious diseases."

Even as vaccine research charges ahead, scientists in the public and private sectors are racing to identify diagnostics, surveillance tools, and antiviral treatments to battle COVID-19—in many cases working to apply existing drugs and technologies to the new health crisis.

Dozens of CSU scientists are in the hunt, Vice President for Research Alan Rudolph said. His office has channeled about $1 million in seed funding to prime COVID-19 projects that hold promise in responding to the pandemic, he said.

"It's a very impactful portfolio of outbreak research and response," said Rudolph, who is working with policymakers, corporate partners, and others to leverage Colorado State's deep expertise in infectious disease.

Adding to its scientific knowledge, the University offers research and development facilities critical to battling emergent infectious disease. These include Biosafety Level-3 laboratory spaces to safely contain potentially dangerous pathogens during research and development; and BioMARC, a manufacturing and testing center capable of producing therapies, vaccines, and diagnostic products in a secure and federally inspected facility.

But well before a vaccine can be manufactured, a candidate must be developed at the laboratory bench. Dean's project begins by examining the intricate process initiated by the SARS-CoV-2 spike protein, which

starts viral infection with the efficiency of a morning star, the medieval weapon with a forbidding spiked ball.

Many vaccine investigations target the coronavirus spike protein. Yet, attempts to activate immunity based on the full length of that spike can risk enhancing infection, rather than halting it.

For this reason, Dean and his collaborators are using CRISPR-Cas9 gene editing technology to modify L. acidophilus, transforming the bacterium into a drug that induces the body's immune response while preventing fusion of the virus to the host cell at two specific sites on the protein spike. The strategy avoids the potential for promoting antibodies that could worsen infection and disease, Dean said.

Vilander analyzes results of polymerase chain reaction by DNA gel electrophoresis. This work at the research bench leads to a vaccine construct for use in investigations. Credit: John Eisele

"We're taking a surgical strike at the spike protein to target these two critical functions, while avoiding parts of the viral protein that are known to elicit the enhancing response," Dean said, while discussing his research at the CSU Regional Biocontainment Laboratory. Here, investigators are working with SARS-CoV-2 in biosecure laboratories.

"Viruses are very clever," he added. "We're always asking, "What are the areas we can hit that they might not be able to respond to?'"

The genetically modified microorganism under investigation is not the same probiotic people seek in yogurt, kombucha, and other fermented foods to support digestion.

Yet, even when altered for use as a drug to combat viral infection, the bacterium offers advantages, Dean said. The vaccine platform is considered safe and could be delivered orally, in the form of a capsule, for instance; it could be inexpensively manufactured and would not require refrigeration, he said.

Just as important, the vaccine could be used to deflect infection by a variety of pathogens—including emergent coronaviruses, such as those that sparked outbreaks of Middle East Respiratory Syndrome in 2012 and Severe Acute Respiratory Syndrome in 2002.

"At CSU, we're trying to tackle this problem from every angle we can with our expertise," he said. "The current situation is dire. I think every scientist who toils in the laboratory hopes that they'll ultimately be able

to have a tangible impact on human or animal health—or both."

Provided by Colorado State University