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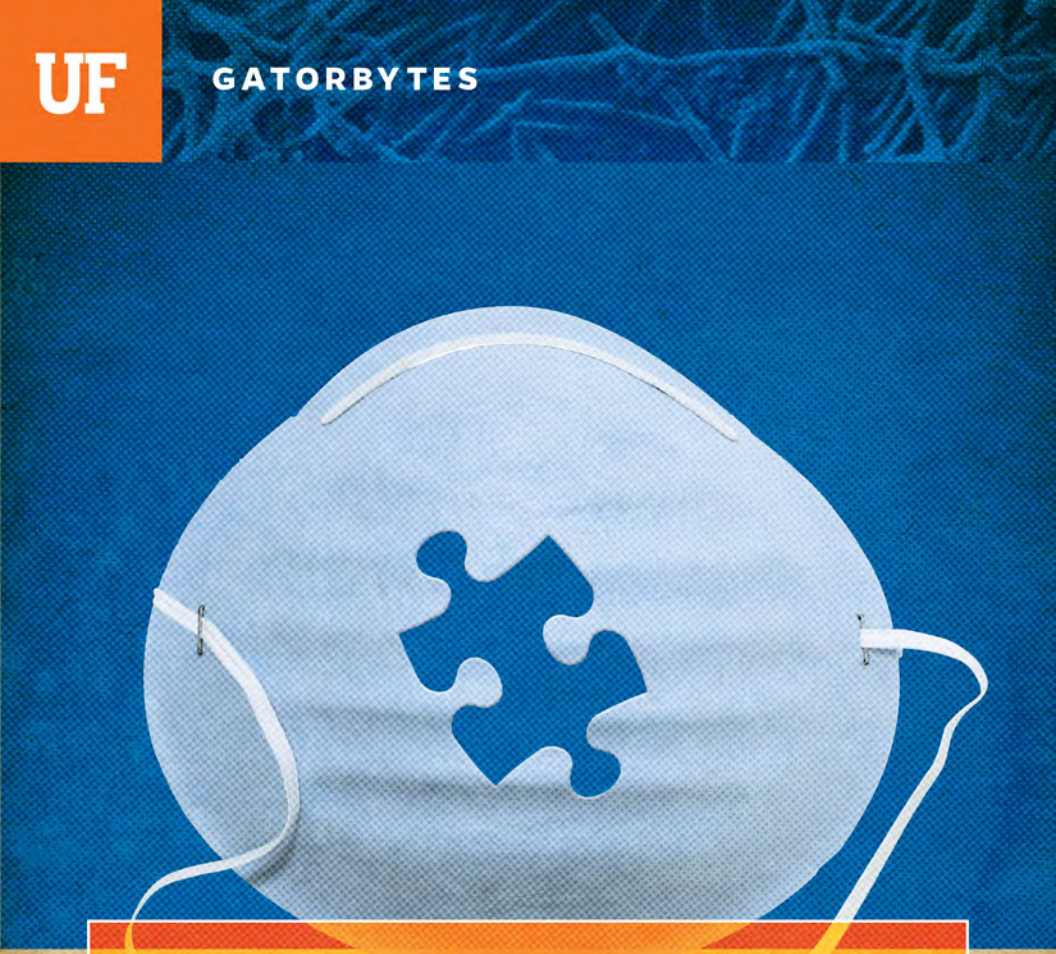

THE DISEASE DETECTIVES

UNRAVELING HOW VIRUSES GO VIRAL

UNIVERSITY of FLORIDA

GATORBYTES

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Unraveling How Viruses Go Viral

Kris Hundley

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Most people think about an infectious disease like the flu only when it knocks them flat.

Or they worry about a deadly virus like Ebola after it shows up in the headlines.

But scientists at the University of Florida's Emerging Pathogens Institute are always anticipating the next possible epidemic, tracking viruses and bacteria that can trigger a fast-moving chain of infection.

Then the team at EPI tries to stop them.

Like scientists before them who devised ways to rid the world of smallpox, EPI's researchers are working on ways to eradicate malaria from Zambia, control cholera in Haiti, and even minimize influenza outbreaks in Alachua County.

Their work involves identifying disease-carrying microorganisms that are constantly evolving, becoming ever more virulent and resistant to treatment.

At the same time, the world that serves as host to these bacteria and viruses is continually changing, its population growing, the opportunities for infection multiplying exponentially.

Dr. J. Glenn Morris, EPI's director and a specialist in infectious disease, said a perfect example of this dynamic is the Ebola crisis in which a virus present in monkeys and fruit bats for thousands of years suddenly crossed over to humans in West Africa.

"Jungles have been cleared and there's increasing pressure from population," Morris said. "Changes in the ways people behave allow the microorganism to spread."

There were more than 19,000 reported Ebola cases and more than 7,000 deaths from the epidemic as of late 2014.

To understand how diseases emerge and are transmitted, EPI's researchers analyze a pathogen's genetic structure in the lab, then create computer models that show how the virus or bacteria might be spread by insects, other animals, or humans. Finally, the scientists at EPI collaborate with colleagues down the hall and around the world to design ways to intervene and block the spread of the disease. No sooner is one puzzle solved than another emerges.

Among the riddles currently being unraveled by EPI's researchers:

How is a pig-borne disease transmitted in a Muslim country?
What kinds of parasites do Florida ticks carry?
How has a cholera strain from Nepal evolved in Haiti?
And when an Ebola vaccine becomes available, who should get it first to have the greatest impact?

Founded in 2006, EPI has about 200 investigators, drawn from 11 different colleges on UF's campus. They work out of a $55 million, four-story facility that's equal parts high-tech labs and high-speed computers. Glass-walled offices house a geographer next to a pediatrician next to an epidemiologist next to an ecologist. Morris, EPI's director since its inception, said the building was deliberately designed to encourage the collaboration needed to move infectious disease research from the lab to the wider world.

"Understanding why and how new pathogens emerge re-

quires a multidisciplinary approach, with the ability to move from basic genetics to global public health," said Morris, who was previously interim dean of the University of Maryland's school of public health. "My goal is to bring faculty together to build a strong interdisciplinary spirit and to play on each other's strengths. If you put them all together in one room and close the door, it is amazing what can happen."

Greg Glass, a professor of medical geography and EPI researcher, puts it this way: "If you only have a bunch of labs, you just wind up mumbling to yourself."

The institute, which reports to UF's vice president of research, has received more than $90 million in grants over the past nine years, with funding from the state of Florida, National Institutes of Health, U.S. Centers for Disease Control and Prevention, U.S. Department of Defense, and the Bill and Melinda Gates Foundation, among others.

Dr. Parker A. Small Jr., a pediatrician and immunologist who came to UF from the National Institutes of Health in the mid-1960s, said EPI has been a game changer at the university.

"It got people on campus talking to each other who hadn't even known the other group existed," he said.

Glass, the geographer who joined EPI's staff in August 2014 from Johns Hopkins University, said he had been on campus only a few weeks when a casual conversation with an agricultural engineer triggered an idea about a possible new strategy for tracking foodborne disease outbreaks.

"He had some interesting approaches to forecasting that have not yet been applied to disease," Glass said. "I'm like a kid in a candy store here."

EPI's researchers have studied pathogens that cause disease in plants, animals, and humans—from salmonella in tomatoes to African swine fever to HIV/AIDS.

They have tracked exotic diseases in far-flung places—Rift Valley fever among goat herders in Kenya and anthrax in Kyrgyzstan.

But they're also working on issues as crucial to Florida's health and economy as influenza, West Nile virus, and citrus greening.

Morris, EPI's director, said the center's holistic approach to infectious disease is applicable whether the outbreak occurs in Kazakhstan or the Florida Keys. Nor does distance matter in a world where pathogens can travel at the speed of a commercial airliner.

"What's going on in Mongolia may be here in a week," he said.

Applying EPI's Strategy to Ebola

That lesson was reinforced during the Ebola epidemic, when a single airline passenger unwittingly carried the virus from Liberia to Texas. Suddenly, a crisis in far-off Africa was front-and-center for the average American.

The scientific community's response to Ebola has also showed how the strategies honed at EPI can be applied regardless of the disease.

Ira M. Longini Jr., a professor of biostatistics at UF, has spent decades constructing and analyzing mathematical models of disease transmission and progression. He has also designed and analyzed vaccine trials for everything from dengue fever to cholera.

So when the World Health Organization was looking for experts to help design upcoming Ebola vaccine trials, they called on Longini, who is now on temporary assignment at WHO headquarters in Geneva.

Longini's assignment: Determine the best ways to evaluate and roll out new Ebola vaccines in West Africa early in 2015.

Here's the challenge: Two different vaccines have been shown in early trials to be safe and trigger an immune response against Ebola in humans, but no one yet knows how effective they will be in the field.

Since time is of the essence, Longini and his colleagues expect to run different trial designs simultaneously to try to identify a winner fast. It typically takes years to determine the effectiveness of a new vaccine; in this case researchers hope to get the answers within months.

In Guinea, which has relatively low rates of infection, Longini expects that frontline hospital and burial workers will get the first vaccinations, along with so-called ring vaccinations of family members and other close contacts of infected patients in an effort to stop spread of the disease.

In Liberia, which has had the highest number of deaths from Ebola, the model will be a classic randomized control trial, with groups of people at risk of exposure selected at random to receive each of the vaccines. Their progress will then be measured against a control group that's been inoculated with a comparison vaccine against some other infectious agent.

In Sierra Leone, randomly selected groups around the country who have exposure risk will receive one of the two vaccines on a staggered schedule, called a *stepped wedge* design. At the first sign that one of the vaccines is effective, the trial will be stopped and the successful vaccine will be given to all participants.

The pressure of working on the Ebola crisis since late August 2014 with colleagues from around the world was wearing on Longini during a brief interview at his office on UF's campus in mid-November. He was headed to Yucatán to help set up a pre-

viously planned dengue vaccination study, then on to Geneva for up to four months to work on Ebola.

"It's been hectic," said Longini, who first became aware of a reappearance of the disease in Guinea early in 2014 but realized there was real trouble in March.

Though there had been about 30 outbreaks of Ebola since 1976, all had occurred in rural, isolated parts of Africa where they were quickly contained. This time, Longini knew, it would be different.

"You've got Ebola in major population centers in countries where poverty and civil wars have weakened the public health structure," he said. "This is the exact nightmare I hoped would not happen."

On the flip side, because of their years of experience working with epidemics, Longini and his colleagues at EPI also know how to put Ebola into perspective. They have advised Florida's congressional delegation on scientifically sound responses to the threat and have recommended against what might seem like sure-fire ways to contain the virus. Closing a nation's borders, indiscriminate quarantining of health-care workers, and widespread airport screening of passengers, they argue, might promote a false sense of security and do more harm than good.

Eben Kenah, an epidemiologist and biostatistician at EPI, remembers crossing the border between India and Bangladesh during the SARS epidemic in 2003.

"A guy on the Bangladeshi side pointed a stethoscope at me and asked me for money," he said. "Officials would rather appear to be doing something rather than nothing."

Kenah, who is studying the potential for transmission of avian influenza among humans in China, said when you design computer models for a living, you develop an intuition for what you don't know. Seemingly strong responses, he has learned, can often backfire.

"Draconian responses to disease outbreaks will trap people in place, encourage people to lie and make it more complicated to get supplies into the affected area," Kenah said. "Sometimes the obvious answer isn't the right one."

Pigs, Ducks, Birds, and a Deadly Virus

Juliet Pulliam, an assistant professor of biology at UF, pulls up a graphic on her office computer at EPI.

On one side it shows a simple diagram of how a specific strain of viral encephalitis traditionally has been transmitted in Japan. A mosquito bites a pig then bites a human, injecting a virus from the pig that can cause severe neurological damage or death.

So how did this pig-borne disease flare up in Bangladesh, a Muslim country where the pig population is minimal?

On the other side of Pulliam's graphic are possible answers: drawings of ducks, pigeons, and chickens that, in lab tests, have been shown to produce the virus.

But those findings trigger a cascade of new questions: What kind of mosquitoes spread Japanese encephalitis in Bangladesh? Why are some people infected but show no symptoms? And, most important, what's the best way to control this disease?

Pulliam, a biologist who was at the National Institutes of Health before coming to UF in 2011, has spent a couple of years trying to untangle the mystery of how Japanese encephalitis is being transmitted in South Asia.

But recently she's been sidetracked by a more pressing issue: Ebola.

In mid-October, she was one of the authors of a letter in the *Lancet*, a leading medical journal, that posed this question: What about the people who are infected with the Ebola virus but do not get sick?

How big is that population? And are these people immune from future infection?

"If you're in public health, you generally don't care about the people who aren't getting sick," Pulliam said. "But the size of that group has implications for how big the epidemic will be. And, if subclinical infection produces protective immunity, you could recruit those people to take care of the sick. You'd create a barrier of people with immunity around the ill, which could protect others from being infected."

Pulliam had worked on Ebola following previous outbreaks in Africa, focusing on strategies to monitor Ebola-related die-offs of wild apes in the Republic of Congo.

More recently, she and the coauthors of the *Lancet* letter reviewed studies from earlier Ebola outbreaks and estimated that somewhere between 20 and 60 percent of Ebola virus infections do not cause clinical illness.

Pulliam said she and her coauthors, led by postdoctoral fellow Steve Bellan at the University of Texas at Austin, wrote the article in part so that people designing clinical trials of the new Ebola vaccines, including EPI colleague Ira Longini, would take this population into consideration.

"We want the clinical trials to include baseline blood sampling—taken before participants are given the vaccine—because it's important to know how many people have been infected and already have antibodies against Ebola virus," Pulliam said.

In a separate interview, Longini was unable to say how issues raised in the *Lancet* letter might affect clinical trial design in Africa.

But Pulliam said the authors have received some interest from researchers in Dallas who are discussing plans to test people there who were exposed to Ebola but showed no symptoms.

Pulliam said being at EPI has given her both the time and the

contacts to pursue questions like the ones raised in the *Lancet* letter.

As a specialist in zoonotic diseases—those that can be passed between animals and humans—Pulliam said EPI offered the ideal combination of experts.

"UF is one of the few places in the country where you have colleges of medicine, veterinary medicine, agriculture, and public health, plus a great basic biology department, right here on one campus," she said. "Everything I've done in my career has drawn on those issues. And the expertise is all right here."

Tick Patrol

Greg Glass is an expert in immunology and infectious diseases who has studied diseases ranging from dengue fever in Brazil to malaria in Zambia. His research has been funded by the Gates Foundation, the National Science Foundation, and NIH, among others.

Glass, just months into his post at EPI, is excited about his next project: making a map of Florida's ticks.

"We're going to track the distribution of ticks in Florida and the pathogens they carry," says Glass. "Everyone thinks Lyme disease and that may be true. But ticks have lots of other microparasites including viruses, so let's go get the ticks and see what's out there."

From invasive South American ticks now found in the Florida Keys to sand-loving dog ticks in the Panhandle, the project will catalogue previously unknown disease threats and propose ways to contain them.

In addition to addressing a compelling public health issue, Glass said the Florida "tick map" will be an ideal way to introduce students to every facet of EPI's work.

"They'll get dirty in the field gathering the ticks, then they'll grind them up and do wet lab work to identify the microorganisms, then they'll do computer modeling showing where people are at most risk for disease," Glass said. "They'll be doing something of immediate value."

Glass knows this approach works because in his previous position at Johns Hopkins, he helped create a computer model that predicted which parts of Baltimore County, Maryland, were at most risk for an outbreak of Lyme disease up to a year in advance. The model took into account weather patterns and geography, including soil types and forested areas, to pinpoint where the black-legged ticks that carry the disease would be most prevalent.

"We were able to show that one area of the county had 20 times the risk of Lyme disease because of the land cover," Glass said. "We could actually tell you the risk household by household."

Hired under UF's Preeminence Plan, funded specially by the Florida Legislature in 2013 to attract high-level talent, Glass is enthusiastic about making the sophisticated research being done at EPI understandable to the general public. In an essay written in 2007, Glass captured the gist of his work with a title even a layperson can understand: "Rainy with a Chance of Plague." Using satellite data to monitor weather, land-use patterns, water quality, and crop conditions, Glass wrote, scientists can forecast viral outbreaks and, most critically, intervene early with vaccines.

Glass knows from experience that researchers' efforts sometimes fall short in the real world.

In the Baltimore tick project, for instance, his model made it possible for the makers of a vaccine against Lyme disease to target likely candidates for inoculation. But the market didn't

turn out to be big enough to sustain the vaccine financially, and it was taken off the market.

A three-year project he participated in to develop a tool to predict dengue fever outbreaks by testing mosquitos for the virus failed. It turned out that the mosquitos could test positive for the virus but not be carrying enough of it to transmit to humans. "No one knew the assays [the tests] were so sensitive," Glass said.

And an effort Glass joined five years ago to restore the public health system in Ukraine was put on hold in February 2014 when Russian troops moved into the country.

"It's the first time I've bumped up against geopolitical realities and seen the consequences on a personal level," said Glass, who was impressed by the resourcefulness of Ukrainian scientists working in difficult conditions. "This job has taught me patience."

These kinds of setbacks—endemic to scientific research—have made Glass a big believer in the collaboration encouraged at EPI.

"As a scientist you're always focused on your paper and your grant," Glass said. "Here you have people working on lots of different problems and willing to share their expertise. It's the quality and quantity of people here that brought me to Florida."

Cholera and Superbugs

Taj Azarian, a PhD student in epidemiology and researcher at EPI, has a bright pink sticky note on the cabinet above his computer. It's a simplified version of a complicated riddle he's been trying to unravel for months.

On the paper, Azarian has drawn three stick figures, each

labeled with a particular strain of cholera found in Haiti. Above the figures are parallel squiggly lines to indicate a waterway, which harbors one of the cholera strains.

Here's the question. If you get rid of cholera in the people of Haiti, but it still survives in the water, is the danger of epidemic over?

Azarian is one of several researchers, including EPI's director as well as scientists in Haiti, who have been working on this issue since 2010. That's when Haiti's catastrophic earthquake destroyed the country's already fragile water and sanitation infrastructure, leading to the first cholera epidemic in Haiti in more than 100 years.

Azarian said researchers were surprised when they analyzed the bacteria from early cholera cases there only to discover it was similar to a strain found in Nepal, nearly 9,000 miles away. "The leading hypothesis is that it came through Nepalese peacekeeping soldiers, who came to Haiti after the quake," Azarian said. "Sanitation was poor, the feces of an infected soldier got into the water and the bacteria took off. It was the perfect storm."

Since then, cholera has infected more than 470,000 Haitians, killing nearly 7,000.

Using samples of the bacteria from both cholera victims and Haiti's waterways, Azarian and his fellow researchers have tracked its continuing evolution through sequencing of its genetic code in the lab. Then they've created computer models showing how the bacteria has mutated over time and been transmitted from human to human.

"There are fewer clinical cases now, but we're still able to recover the bacteria from the water," Azarian said. "The question is will Haiti be able to eradicate the disease or will the environment serve as a reservoir for future outbreaks?"

The answer, he and other researchers hope, will help Haitian officials better target limited resources in their fight against the disease. Among the efforts being fostered through collaboration between EPI and the Haitian government is improved monitoring of wells that were built by foreign aid groups after the 2010 earthquake but often left untended.

"The easiest thing for an outside group to do is build wells, then move on," Azarian said. "We need to know what type of contamination is in those wells."

Azarian, who got his master's in public health at UF, returned to the campus for his advanced degree in 2011. He had been working at the Florida Department of Health in Duval County when EPI researchers came to investigate the spread of the antibiotic resistant strain of bacteria known as MRSA (Methicillin-resistant Staphylococcus aureus) among several Jacksonville-area hospitals.

According to the Centers for Disease Control, MRSA is the most prevalent of about twenty different kinds of "superbugs," responsible for more than 80,000 infections and 11,000 deaths in the United States each year.

Now, working with mentors at EPI as well as UF's colleges of medicine and public health, Azarian is tracking MRSA transmission in neonatal units of Jacksonville hospitals back into the community to figure out how to prevent outbreaks.

His detective work starts in the lab, analyzing samples of the bacteria, which mutates as it reproduces, to identify the relationship between strains.

"We didn't want to just know they were all 'Smiths' or even 'John Smiths,'" Azarian said of the task of breaking apart the bacteria's DNA to track changes in its genetic code over time. "We wanted to identify it down to its social security number or even fingerprint. Then we create a family tree, known as a

phylogeny, to show how the different strains are related to each other. This ultimately allows us to understand how the bacteria is being spread."

Through his research, Azarian found that in three of seventeen cases originally suspected to part of the same outbreak in neonates, the MRSA strains were closely related, indicating a common source of infection.

Now he's digging into those patients' medical records, staffing patterns of the neonatal unit and even the location of the infants' bassinets to trace how the related MRSA strains were introduced to the ward. Most likely, he says, they are being introduced from patients, visitors, and family members who innocuously harbor the bacteria and unsuspectingly spread them to neonates who are highly susceptible to severe infections. This points the finger at a larger, unchecked epidemic in the community at large. To investigate the factors contributing to this community-wide epidemic, Azarian has turned to the overarching issue of antibiotic misuse.

By analyzing 24 months of Florida data on retail and mail-order antibiotic prescriptions, he learned that Floridians fill an average of 50 million antibiotic prescriptions a year. Children ages one to four had the highest rate of antibiotic prescriptions, nearly double the state average, equating to two antibiotic prescriptions per child per year. Additionally, certain counties in north Florida had higher-than-average prescribing rates.

Considering that previous research conducted by the CDC has shown that up to half of all antibiotics are unnecessarily prescribed, Azarian said these rates are alarming.

"The hypothesis is that there's an association between the rates of antibiotic prescribing and the prevalence of antibiotic-resistant bacteria in a community," said Azarian, who expects to complete his dissertation on MRSA in 2015, then seek a post-doctoral research fellowship. "My biggest message is that we

need to look at what's driving the emergence of MRSA and reduce unwarranted antibiotic use."

Stopping the Flu at Home

In the 1980s, long before he was designing trials for Ebola vaccines, EPI's Ira Longini created a computer model that predicted if a community simply vaccinated 70 percent of its school children against influenza, it could sharply reduce seasonal flu outbreaks.

It was the same thinking that had led to the eradication of smallpox: You don't have to inoculate everyone, just a critical mass of those most likely to transmit the disease, in order to have a significant impact.

Unlike smallpox, however, since the flu virus lives in birds it can never be completely eradicated, just controlled. But immunize enough school children to prevent them from carrying the flu virus home to siblings, parents, and grandparents, Longini's model showed, and outbreaks could be minimized.

As sensible as it sounds, the idea has yet to gain widespread acceptance in the United States. For most Americans, the flu shots advertised by pharmacies are for old people, not kids.

But that may be changing, thanks to a school-located flu vaccination program led by a team of EPI researchers in collaboration with Alachua County health and school officials and local pediatricians. Over the past four years, the effort has helped raise the vaccination rate among the county's school-age population to 50 percent, significantly improving the community's health.

The program was started in 2006 with a grant from Children's Miracle Network and free vaccine from MedImmune.

In 2009, EPI's director recruited Cuc Tran, a doctoral student in public health, to run the program and build community sup-

port. Tran reached out to dozens of community groups and local media, explaining the scientific basis for the project. Dr. Kathleen Ryan, a UF pediatrician and EPI researcher, worked to coordinate the program with pediatricians in the community.

In an effort to increase participation rates, the EPI team, including volunteers from the UF School of Nursing, spend countless hours every year at back-to-school fairs and school car lines, talking to parents and encouraging them to get their children vaccinated. To get the youngest kids excited about the program, EPI interns go to elementary schools dressed in bright green and orange flu costumes (think bulbous, spiky, and ugly). At middle schools, volunteers use popsicles and promises of pizza parties to bribe students to return parental consent forms.

The inoculations, using a nasal spray vaccine provided by the county health department, take place in October, with the program vaccinating as many as 1,500 children in a single day.

Approximate cost is $375,000 a year or about $26.50 in vaccine and administrative costs per student, part of which is reimbursed by insurance companies. The program also receives $100,000 a year from Alachua County and about $5,000 a year from AvMed Health Plans to defray costs.

Alachua County's vaccination rates now are considerably higher than the national average: close to 65 percent among elementary school students, 50 percent in middle school, and 30 percent at the high school level. By comparison, published reports show vaccination rates among privately insured children nationwide at 31 percent for five- to eight-year-olds and 18 percent for the nine- to seventeen-year-old age group.

Working with her advisors at EPI, Tran recently had a paper published in *PLOS ONE*, an open access medical journal, on the effectiveness of the program. It compares the number of flu cases in Alachua County for the past two seasons with the rest of Florida.

The findings: While vaccinating school-aged children didn't have a big impact on the number of people over 65 who came down with the flu, it made a big difference for the youngest residents of the county.

Rates of infection for children up to age four, a particularly vulnerable age group, were 89 percent lower in Alachua County than in the rest of the state.

"That's pretty profound," Tran said.

EPI's researchers suspect the failure to have much effect on flu rates among the elderly may be due to the high concentration of college students in the area. This group, which typically has low vaccination rates, may be spreading the flu virus to the over-sixty-five crowd at restaurants, malls, and movie theaters.

Next up for EPI's flu vaccination project: target college students for the nasal spray vaccine as well.

Tran hopes the *PLOS ONE* article sparks more discussion of school vaccination programs in the United States and better coordination among the handful of existing programs around the country. She notes that the United Kingdom recently announced plans to expand its flu vaccination program to include healthy school children based on the result of computer models and the data published so far. In order to gain traction for a similar movement in the United States, Tran and her colleagues are eager to share what's been learned in Alachua County, whether it's with public health departments or private companies that want to get into the school vaccination business.

"I'm not concerned about who does it," said Dr. Parker A. Small Jr., an EPI researcher and longtime flu expert who advised Tran. "I just want them to do it well."

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