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Frozen assets: Researchers turn to unique resource for clues to norovirus evolution

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A search through decades-old frozen infant stool samples has yielded rich dividends for scientists from the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health. The team customized a laboratory technique to screen thousands of samples for norovirus, a major cause of acute gastroenteritis outbreaks in people of all ages. What they discovered about the rate of evolution of a specific group of noroviruses could help researchers develop specific antiviral drugs and, potentially, a vaccine against a disease that is very unpleasant and sometimes deadly.

The research, led by Kim Y. Green, Ph.D., and Karin Bok, Ph.D., of NIAID's Laboratory of Infectious Diseases, will appear in a future issue of the *Journal of Virology*, and is now available online. NIAID scientist Albert Z. Kapikian, M.D., is a co-author on the paper. In 1972, Dr. Kapikian and colleagues identified and characterized the virus, now known as norovirus, responsible for an outbreak of acute gastroenteritis in Norwalk, Ohio, in 1968.

"Thanks to the foresight of Dr. Kapikian and others at NIAID and the Children's National Medical Center who established and have maintained these clinical samples since 1974, our researchers have a unique resource that represents one of the oldest sets of norovirus samples in the world," says NIAID Director Anthony S. Fauci, M.D. "This is the first study to look at samples that date back almost to the first recorded cases of norovirus outbreaks, more than 40 years ago."

Highly contagious, noroviruses are responsible for an abrupt onset intestinal ailment also called winter vomiting disease or cruise-ship disease. The Centers for Disease Control and Prevention (CDC) estimates that 23 million cases of acute gastroenteritis each year are due to norovirus infection and that noroviruses are the cause of more than half of all foodborne gastroenteritis outbreaks. In elderly people, infants and people with compromised immune system function, dehydration resulting from vomiting and diarrhea following norovirus infection can be life-threatening. In developing countries, according to a 2008 estimate by CDC researchers, up to 200,000 children under 5 years old die of norovirus infection each year. There is no vaccine against norovirus and no specific antiviral drugs to treat infections.

A key question for norovirus researchers is determining when a dominant variant, called genotype II.4 (or GII.4), first emerged, notes Dr. Green. "This genotype has been associated with the majority of global outbreaks of acute norovirus gastroenteritis since the mid-1990s," says Dr. Green. "The GII.4 genotype was first described around 1987, but no one knew for sure whether that genotype emerged then or if it existed earlier."

To answer the question, Dr. Bok customized a new technique—real-time reverse transcriptase-polymerase chain reaction (RT-PCR)—and applied it to stool samples originally collected from infants and young children hospitalized at the Children's National Medical Center in Washington, D.C., between 1974 and 1991. Samples were taken from infants and children with gastroenteritis and from others (controls) who did not have gastroenteritis. Essentially, Dr. Bok crafted genetic hooks capable of fishing out matching genetic sequences of any norovirus present in the samples. Fifty out of 5,424 samples tested contained norovirus. The most commonly seen genotype was GII.3 (48 percent), but the second most common genotype was GII.4 (16 percent). Some GII.4-containing specimens dated back to 1974, allowing the researchers to conclude that

this now-dominant genotype had been circulating for years before its more recent identification as the cause of severe global outbreaks of norovirus disease.

Next, using a strategy developed by NIAID scientist Stanislav Sosnovtsev, Ph.D., the researchers determined the complete genetic sequences of five older GII.4 viruses and compared those sequences to gene sequences of contemporary GII.4 noroviruses. The comparison allowed the investigators to determine how much the archival viruses differed from the most recent representatives of the same genotype and, thus, to calculate how quickly the GII.4 genotype is evolving.

Currently, there are no antiviral drugs specifically targeted to noroviruses, but the new knowledge about which segments of the norovirus genome change the least could aid in the development of novel drugs that could be targeted at those more genetically static portions of the virus, say the researchers. Noroviruses, like influenza viruses, mutate readily and evolve rapidly, explains Dr. Green. If vaccines against noroviruses become possible in the future, researchers would need to take into account shifts in the virus's genetic make-up and reformulate the vaccines to match the virus, she adds. However, unlike influenza viruses, noroviruses cannot be grown in the lab, raising an additional hurdle to vaccine development.

"By examining the history of norovirus evolution contained within these archival samples, we can see how the virus has changed during this time, and we also can better predict how the virus is likely to change in the future," says Dr. Bok. If scientists one day crack the problem of growing norovirus in the lab, information about the rate of evolution will be invaluable to developing vaccines, adds Dr. Green.

"This research is the first to reveal the speed at which the molecular clock of norovirus runs," says Dr. Green. Dr. Green and her colleagues

are now looking at stool samples from the 1960s in Dr. Kapikian's collection. If norovirus can be detected in those samples, knowledge about the ancestry and rate of evolution of this virus will be further expanded.

More information: Additional information about noroviruses is available from NIAID at www3.niaid.nih.gov/topics/norovirus/ and from the CDC at www.cdc.gov/ncidod/dvrd/revb/g … ovirus-factsheet.htm

• K Bok et al. Evolutionary dynamics of GII.4 noroviruses over a thirty-four year period. Journal of Virology. DOI: 10.1128/jvi.00864-09

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