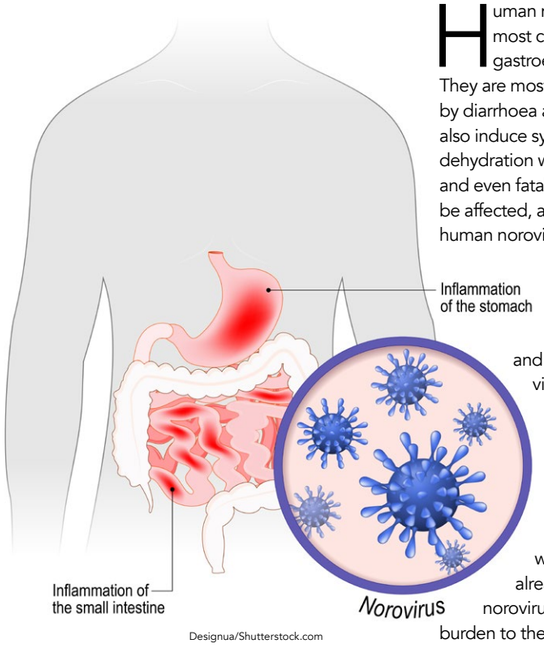
Human norovirus animal model essential for vaccine development

Human noroviruses are the most common cause of acute gastroenteritis and represent an incredibly high burden on the healthcare sector. Currently no vaccines or drugs exist to prevent or treat the disease. This is in part due to a lack of animal models. Here, Dr Lijuan Yuan and the team at Virginia Polytechnic Institute and State University developed and validated gnotobiotic pigs as an animal model for the human norovirus GII.4/2003 Cin-2. Determining dose-response relationships as well as the median infectious dose, by using different statistical approaches for this virus, means we are one step closer on the path to vaccine development.



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Human noroviruses are the most common cause of acute gastroenteritis around the world. They are most frequently characterised by diarrhoea and vomiting but can also induce symptoms of fever and dehydration which can be dangerous and even fatal. People of all ages can be affected, and it is estimated that human noroviruses cause 125 million foodborne illnesses annually. This incredibly high number is due to the highly transmissible and infectious nature of the viruses as well as their long-lived stability in many environments. This is also why these viruses often cause outbreaks in densely packed sectors such as schools as well as hospitals where patient health is already compromised. Human noroviruses represent a significant burden to the healthcare industry as well as having an enormous financial cost. Despite this, there are no norovirus vaccines to prevent the disease or any anti-viral medicines to treat it.

THE IMPORTANCE OF ANIMAL MODELS

Before being accepted for human clinical trials, antivirals, antibodies, and vaccines need to first demonstrate their effectiveness in animal models. The lack of an animal model for studying human norovirus infection and disease has delayed the development of anti-norovirus drugs and vaccines. However, it is now known that gnotobiotic pigs make excellent human norovirus animal models. Gnotobiotic pigs are raised in sterile, germ-free environments so all microbe exposures can be accounted for. This means that, if infected with an organism, it's clear that any symptoms that develop will have stemmed from this. These pigs are good candidates as animal models due to their shared similarities with humans. They have similar immune responses as well as virus-binding patterns. This means that disease progression and infection doses determined from these pigs are comparable to what would occur in humans.

A key factor that can be learnt from these animal models is the median infectious dose. This is the amount (or dose) of the virus that must be ingested for 50% of the population to become infected in the animal-model study. The median infectious dose can be determined using various statistical methods. Classical methods can provide an estimate, but more contemporary mechanistic dose-response models may prove to be more effective and accurate. These newer methods can also give an overall picture about infection at any dose level, not just 50%. Once this has been established, the median infectious dose can then be used as a starting point for human pre-clinical studies or risk assessments to do with food and water safety. Since the results between animals and humans is comparable, this ensures safety for testing new drugs without exposing people to high levels of the virus.

The researchers study human noroviruses using gnotobiotic pigs.

HUMAN NOROVIRUS STUDIES USING GNOTOBIOTIC PIGS

Dr Lijuan Yuan and the team at Virginia Polytechnic Institute and State University have been carrying out studies on human noroviruses in gnotobiotic pigs for many years. They established the gnotobiotic pig model of human norovirus infection and diarrhoea for vaccine evaluation. They have also carried out experiments to test changes in infection and immunity in response to various factors. It has been discovered that probiotics provide protection against norovirus while the drug simvastatin enhances the infection. They have also looked into the relationship between noroviruses and the human gut microbiota by transferring human gut bacteria to these gnotobiotic pigs.

Their most recent study, however, focuses on determining the median infectious dose and the median diarrhoea dose with a pandemic strain of human norovirus known as GII.4/2003, or Cin-2. The median diarrhoea dose is the amount of virus that causes 50% of the animals to develop diarrhoea after infection. In order to determine these numbers, the gnotobiotic pigs were separated into seven different groups with each group being inoculated with a different dilution or dose of the virus. Samples were then taken every day from the animals and analysed for viral RNA that had been shed. Diarrhoea symptoms were also assessed daily for seven days.

Human noroviruses represent a significant burden to global health as well as having an enormous financial cost.

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Noroviruses are a huge public health burden.

Research using animal models brings us closer to a vaccine.

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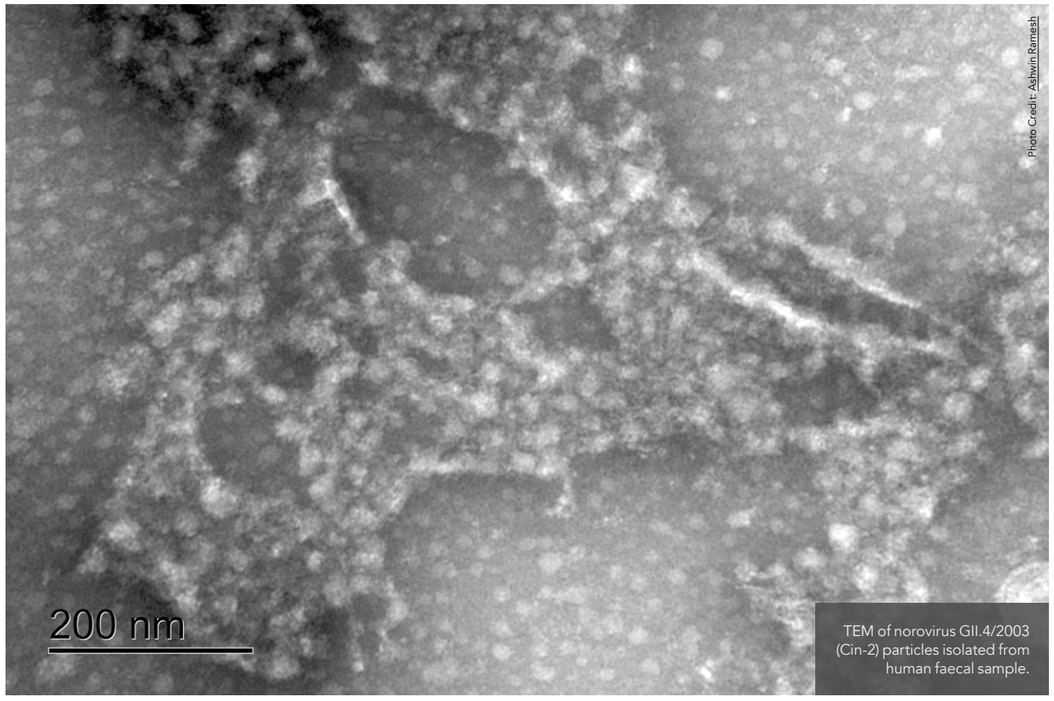
ESTABLISHING THE DOSE-RESPONSE RELATIONSHIP

From these experiments it was determined, as expected, that higher doses of virus had shorter incubation periods, so animals were infected more quickly. Infection persisted in animals infected with higher doses for longer, and shed higher amounts of virus overall in the study than the animals given lower doses. Similarly, the diarrhoea symptoms appeared earlier and for longer in the animals given a higher dose of virus. Pigs given a dose of 2×10^5 RNA copies of viral genomes had the highest and longest level of viral shedding and diarrhoea symptoms. On average they were shedding viral particles for 6.3 out of the 7 days and diarrhoea symptoms began 2.8 days after inoculation lasting for 4 days. This makes 2×10^5 the optimal challenge dose for this GII.4/2003 human norovirus. This was the third-highest dose of all the groups. These results were similar to those from a previous human volunteer challenge study, showing again that the gnotobiotic



TEM of norovirus GII.4/2003 (Cin-2) particles isolated from human faecal sample.

pigs are a replicable animal model in humans for these noroviruses.

Various calculations were used to work out the median infectious dose and the median diarrhoea dose. Classical methods can be useful in these experiments, but they were developed before more accessible computer access, so they do have some shortcomings. The Reed–Muench method gives an idea of dose response, but the doses administered to the animals need to be equally spaced logarithmically and the number of animals has to be the same in each group tested. The Spearman–Karber test, on the other hand, has the drawback of needing a 0% and a 100% response in order to develop a dose-response curve. In some cases, this isn't possible; for example, pigs in each group shed viral particles so there was no 0% response. Exponential and beta–Poisson were more contemporary methods for endpoint estimation used to analyse results. These give more accurate and flexible estimations. Using all of these calculations it was determined that the median infectious dose was between $2.4x\ 10^3$ and $3.4x\ 10^3$ RNA copies of viral genomes while the median diarrhoea dose was between $2.1x\ 10^4$ and $3.8x\ 10^4$ RNA copies of viral genomes.

It is worth noting that all these analyses were carried out with the assumption that the viral particles were not aggregated. However, it has been seen in previous studies in which other human noroviruses were visualised by electron microscopy that they are often aggregated. The viral particles are assembled within membrane-bound vesicles which gives them more stability and means they can survive longer. Electron microscopy was therefore carried out on the noroviruses used in these experiments and confirmed that they were indeed aggregating. This means that the median doses determined were for aggregated particles and it can be expected that disaggregated viral particles would give lower median infectious and diarrhoea doses.

These experiments have shown that infection and disease caused by human noroviruses in gnotobiotic pigs resemble those seen in humans.

THE PATH TO VACCINE DEVELOPMENT

These experiments have shown again that responses to human noroviruses in gnotobiotic pigs are comparable to the responses seen in humans. Disease presents and develops in the same way. Analysing dose-response relationships using various methods leads to a better understanding of how human noroviruses cause disease. Knowing the full extent of how much virus is needed to cause disease and induce symptoms means that an optimal dose can be worked out for testing novel vaccine candidates. The development of vaccines and drugs against human noroviruses is essential to prevent and treat the disease, and reduce the burden it puts on the healthcare sector globally.

Behind the Research

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Research Objectives

Dr Lijuan Yuan and team investigate the use of gnotobiotic pigs as an animal model for the human norovirus GII.4/2003 Cin-2.

Detail

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Dr Lijuan Yuan is a professor of virology and immunology at Virginia Tech. Ashwin Ramesh was a PhD student in Yuan Lab. Dr Viviana Parreno, an investigator at INTA-CONICET, Argentina, analysed data to obtain her master's degree in biometrics. Currently, she is a visiting research scientist in Yuan Lab.

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Collaborators
- Xi Jiang from Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA for providing the HuNoV GII.4 inoculum pool.
- Philip J Schmidt and Monica B Emelko from Department of Civil & Environmental Engineering, University of Waterloo, Waterloo, Canada, for contributions to statistical analyses.

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Personal Response

How close do you think we are to the successful development of a human norovirus vaccine?

"We are working with collaborators in efforts to speed up the development of human norovirus vaccines. A quadrivalent virus-like particle vaccine developed by Anhui Zhifei Longcom Biologic and evaluated in our pig model is currently under a phase I/IIa human clinical trial (NCT04563533). An oral norovirus GI.1 vaccine based on Ad5 vector is under phase 1b clinical trial (NCT04854746). There are many other ongoing efforts. A successful vaccine is possible within the next 5 years."

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