Sequencing Tracks a Deadly Porcine Virus

The MiSeq® system is enabling veterinary scientists to characterize an emerging porcine virus, and track its molecular evolution and spread.

Introduction

In the early 1970s, an English pig farmer discovered something was amiss with his swine herd. Young piglets were dying off quickly from an illness that looked like transmissible gastroenteritis virus (TGEv) infection. In 1978, veterinary scientists discovered that the deadly disease was caused by a coronavirus, subsequently named the porcine epidemic diarrhea virus (PEDv). PEDv spreads easily through oral-fecal contact with infected feces and manure contamination of fomites that come into contact with swine.

Until recently, PEDv was only found in Europe and parts of Asia. But in April 2013, the virus was discovered on pig farms in Iowa and Indiana.[1] Since then, millions of piglets have succumbed to the disease—with 100% mortality for newborns on some swine farms. Those deaths have played a key part in the substantial increase in pork prices across the globe. In the past few months, pork prices have reached record highs in the US, in large part due to the estimated 10% death loss among herds in the past year. Despite the ongoing epidemic, scientists remain unclear on just how the disease entered the US, how it may be mutating, and how to stop the spread from herd to herd across the nation.

Shortly after the disease was identified in US swine, a team at Iowa State University's Veterinary Diagnostic Laboratory (ISU-VDL) at the College of Veterinary Medicine isolated and sequenced PEDv in the hopes of determining which of its genes might be suitable for tracking its evolution.[2,3] Their findings may hold the key to developing vaccines and preventing PEDv deaths in the future. Recently, two companies, Harrisvaccines[4] and Zoetis[5], received conditional licenses by the United States Department of Agriculture (USDA) for PEDv vaccines. Preliminary studies for both products have been hailed as "promising," however, no hard data are yet available—and it is unclear whether they will remain as effective once winter comes, as the disease is known to flourish in colder temperatures.[6]

iCommunity spoke with Ganwu Li, Ph.D., an Assistant Professor in the Department of Veterinary Diagnostic and Production Animal Medicine, and the ISU-VDL about the risk of PEDv to the American pork industry and how next-generation sequencing (NGS) may help scientists finally develop an effective PEDv vaccine.

Ganwu Li, Ph.D., is an Assistant Professor in the Department of Veterinary Diagnostic and Production Animal Medicine and the Veterinary Diagnostic Laboratory (VDL) at Iowa State University's College of Veterinary Medicine.

Q: What is the main research focus of your lab?

Ganwu Li (GL): We focus on diseases of production animals, like pigs, and look at their effects on animal health, public health, and food safety. As part of that goal, we use techniques like NGS to characterize known pathogens and detect new viral variants or novel pathogens.

Q: How did the lab become interested in PEDv?

GL: This virus is new to the US and the swine industry. The ISU-VDL was the first lab to diagnose PED in US swine. Since the initial diagnosis, it has caused serious problems and economic losses in the US pork industry. The virus results in severe diarrhea in young pigs, which has led to high mortality rates close to 100% in naïve populations and significant economic loss to the pork industry over the past year. The ISU-VDL has demonstrated a long history serving the US swine industry and its stakeholders, so it was natural that it would be involved in such an economically significant disease.

Q: Is this virus a threat to human beings?

GL: Luckily, no. It is not a threat to people. At this time, we know it is harmful to pigs, especially to very young pigs, although infection in other species has not been thoroughly investigated.

More about PEDv

PEDv was first discovered in the United Kingdom in 1971. The National Hog Farmer states that one thimble-full of feces could contain enough of the virus to infect all of the pigs in the United States.[6] PEDv invades the cells that line the animal's small intestine, resulting in its hallmark clinical signs (diarrhea)—and may be fatal within five days of transmission for suckling piglets.

The disease spreads easily. Adult pigs most commonly develop the disease by oral-fecal contact, with piglets at a significant risk for this contact while nursing. Lax biosecurity control measures enable the spread of the virus, as infected manure contaminates work boots, farm equipment, transport trucks, and packing plant equipment. This is how PEDv and other viruses can move quickly from farm to farm and why the disease has traveled so far so quickly.

Q: For a long time, PEDv was only present in Europe and Asia. Last year, it spread to the United States. How are we tracking the spread of this virus?

GL: Initially, scientists used polymerase chain reaction (PCR) techniques, a biochemical technology that can amplify DNA and RNA sequences of viral genome, to confirm the presence of the virus. By this means, the spread of PEDv in US swine herds was tracked. Later on, sequencing selective genes of PEDv, like the spike gene, was performed. Phylogenetic analysis of the sequences helped determine the genetic relationship of virus strains and helped track where the virus came from and where it might be going.

NGS represents a game-changing opportunity in virus diagnostic practices. Whole-genome viral epidemiology is the ultimate source for this information and will not be superseded.

The ISU-VDL was the first lab that diagnosed PED and isolated the virus in the US. My colleagues, Drs. Kyoung-Jin Yoon and Jianqiang Zhang led the groups that respectively detected and successfully isolated the virus and then I did the whole-genome sequencing on the isolates. By using NGS, we were also able to sequence the whole genome directly from clinical samples like feces and intestines. Compared to one specific gene sequence, whole-genome sequencing can provide additional information to more thoroughly characterize the genetic PEDv profile and to better track spread of the virus.

Q: Many in the pig farming industry are becoming increasingly nervous about how this disease is spreading. How will your research help track PEDv?

GL: We have performed PEDv whole-genome sequencing from more than 30 samples submitted from across the country and confirmed that there are at least two different viral strains circulating in the field. Like any other RNA virus, PEDv can be always changing and generating genetic and antigenic diversity. So, it is important to monitor these changes and see how fast the virus changes. It is proposed that we assess 12-15 PEDv whole-genome sequences each month to trace how this virus is changing. If we determine that a new variant has emerged, we need to find out if it can be prevented by the existing immunity in the swine population or current vaccines, or if we need to develop a new vaccine. As a result, our whole-genome sequences can help veterinarians understand if a different strain of PEDv has infected a herd and also provide guidance for the development of new vaccines.

> "NGS technology makes it possible to quickly determine the whole-genome sequences of emerging, previously unrecognized, or new pathogens."

Q: How might a greater understanding of the PEDv genome enable us to better combat the disease in swine herds?

GL: A better understanding of the PEDv genome will allow us to identify and investigate genetic diversity that may be the result of mutations and genomic recombination and contribute to increased or decreased virulence, antigenic variation and loss of protection from existing immunity or vaccine immunity. Understanding the PEDv genome more thoroughly also allows the development of novel diagnostic assays to detect and differentiate various virus strains. With whole-genome sequencing, we may discover clues that can help devise more effective strategies to identify, monitor and combat this disease.

Q: How might whole-genome sequencing inform vaccine development?

GL: In general, NGS technology makes it possible to quickly determine the whole-genome sequences of emerging, previously unrecognized or new pathogens. The whole genome sequences can reveal the genetic diversity of the viruses that may be helpful to develop appropriate vaccines against diverse viruses. In addition, quick determination of the whole-genome sequences of viruses with different phenotypes, eg virulent and attenuated, and/or antigenic variation will help determine the genetic changes responsible for the phenotypic differences. Therefore, I believe that the increased functional genomic knowledge of PEDv will ultimately lead to better characterization of the virus and decision making for intervention.

Q: Have you used NGS before in your research?
GL: I am a bacteriologist by training, and in the past I have done a lot of bacterial pathogenesis work. I only started the research project involving viruses two years ago. Since then, we have used NGS to sequence the whole genome for PEDv, and other viruses like the porcine reproductive and respiratory syndrome virus (PRRSV) that causes reproductive failure in breeding stock, influenza A viruses in swine, influenza D viruses in swine and cattle, and the porcine deltacoronavirus. We continue to expand our usage of NGS technology to other pathogens to be ready for emerging or new pathogens.[7]

Q: What kind of system did you use to perform the whole-genome sequencing?
GL: Iowa State University has the Illumina MiSeq System in its core laboratory among other platforms available for NGS. It enabled us to determine entire genome sequences quickly and at a lower cost. We used the TruSeq® Library Preparation kit to prepare our samples.

> "It (the MiSeq System) enabled us to determine entire genome sequences quickly and at a lower cost."

Q: What were the steps you followed in your study?
GL: We started by sequencing the viral genome from cell culture isolates. These are relatively pure. Then we went on to our clinical samples. Those are not as pure as virus isolates, so we had to modify the standard library preparation protocol. We used a cleaning procedure, which involves a centrifuge to remove most of the host contamination and an ultracentrifuge to enrich the viral particles and then isolate the viral RNA. After doing that, we used a standard protocol from a commercial kit to prepare the sequencing library to sequence viral genome.

Q: What software did you use to analyze the MiSeq data?
GL: We developed our own pipeline to assemble the whole viral genome. We had to use BWA to map the reference sequences employing very relaxed criteria for mapping and then used the ABySS software to perform *de novo* assembly. By combining all of these steps, we were able to obtain the entire genome sequence of the virus.

Q: In the past, other labs have not been as successful in isolating PEDv. What do you think was the secret to your lab's success?
GL: There are really no secrets to how to isolate PEDv in cell culture. We have followed the published procedures. The success rate in isolating enteric viruses is notoriously quite low. You have to attempt isolation from a large number of clinical samples in order to have a better chance of successfully isolating the virus. We are continuing to look for a better system to increase the isolation rate of PEDv.

Q: What are the next steps in your research?
GL: We are very interested in using NGS as a better way to study the diagnostics, epidemiology, pathogenesis, and evolution of PEDv, as well as other pathogens of veterinary significance, and its impact on diagnostics, immunity and pathogenesis.

I am also interested in using NGS to look at how the microbiota may affect diseases or vice versa. For example, with PEDv, I would like to see how different composites of bacteria in the gastrointestinal tract may affect the disease development as well as response to treatment or immunization, and how PEDv infection may alter gut microbiota.

References

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