

TRACKING *Salmonella's* EVOLUTION FROM INNOCUOUS TO VIRULENT

STEPHEN AUSMUS (D1335-16)



To better understand how bacteria evolved the ability to get into eggs, Jean Guard-Bouldin and technician Cesar Morales analyze how many colony types are present in the organs of chickens infected with *Salmonella*.

In forensic science, DNA is often the last word. But an ARS scientist has found that even having one full DNA sequence may not be the definitive answer in the case of *Salmonella* microbes.

A team of researchers led by veterinary medical officer Jean Guard-Bouldin at ARS's Egg Safety and Quality Research Unit in Athens, Georgia, has found *S. enteritidis* strains to be so similar genetically that they appear identical—yet they may behave differently inside the hen.

To distinguish between the apparently identical genomes, researchers must use a technique called “whole-genome mutational mapping” to analyze multiple strains. It's a way of comparing the genomes to find small differences in DNA—called “polymorphisms”—among them.

In food safety and public health, *S. enteritidis* is problematic.

It is the only *Salmonella* pathogen that is found inside the egg, is present in healthy hens, and is able to cause illness in humans.

“To reduce current levels of infection, we're studying how *S. enteritidis* evolves and infects hens on the farm,” says Guard-Bouldin. “Using mutational changes in the *Salmonella* genome as a sort of ‘breadcrumb trail,’ we've tried to determine the first time this bacterium became capable of getting inside the egg from hen reproductive organs.”

Mapping Mutations

The mutational maps for *S. enteritidis* were obtained by using a database from the Sanger Institute to generate a set of primers that “tile” the DNA of the test strains. Tiling keeps fluorescent-labeled DNA from producing light as a signal. If a signal is produced, that means that a mismatch is present in the test strain. All potential mismatches, or polymorphisms, are sequenced multiple times in multiple strains to identify the exact location and nature of the change. To date, 447 polymorphisms have been detected, and about half of these have been confirmed by sequencing.

Down the Genome Path

“*S. enteritidis*, like a lot of other bacteria, is able to reproduce very quickly—in optimal conditions, every 20 minutes,” says Guard-Bouldin. “Such a fast pace allows the organism to accumulate polymorphisms. Only healthy competitors go on to reproduce, survive, and then thrive in the infection pathway to the egg.”

Once her mutational maps were complete, Guard-Bouldin could apply a mathematical approach that compares the number of polymorphisms seen today between strains that have been recently isolated to come up with a rate of evolution. This has led to a theory that a large-scale swap of DNA about 36 years ago occurred between strains in association with the emergence of egg contamination. Evolution beginning hundreds of years ago, however, probably laid the footing for today's problem.

While the hybrid strain that recently emerged had the ability to contaminate the internal contents of eggs, it also had a problem: It was carrying incompatible viruses within its genome.

As a result, says Guard-Bouldin, “The hybrid strain split very quickly into two lineages, each carrying one virus. Except for the different viruses, the two strains had identical genomes, and both contributed to the beginning of the pandemic. Both of the newly split lineages continued to evolve by accumulating small changes in their genomes. They eventually began to vary in their ability to contaminate eggs, to survive on the farm, and to challenge our ability to understand their association with chickens.”



A typical egg contamination experiment often involves as many as 1,000 eggs. Here, Jean Guard-Bouldin removes the contents from eggs, which are then added to liquid media and cultured for the presence of *Salmonella*.

Searching for Small Changes

Paula Cray, research leader of ARS's Bacterial Epidemiology and Antimicrobial Resistance Research Unit, also in Athens, was able to detect a few of these changes using a more conventional method—pulse field gel electrophoresis (PFGE). This method provided a baseline for estimating the frequency at which DNA changes were being missed. Whereas mutational mapping found 447 polymorphisms, 12 of which disrupted genes, PFGE found only 2 differences between the *Salmonella* strains that varied in the ability to contaminate eggs.

“In fact, in a comparison of PFGE patterns, every band difference could account for about 200 polymorphisms going undetected,” says Guard-Bouldin. Cray's research is a good example of why food safety agencies are continuously testing which methods are best for detecting evolutionary trends in bacteria that threaten public health.

The New Horizon

The data from this research is being entered into a publicly available database by the National Center for Biotechnology Information, part of the National Institutes of Health.



An important intermediate step in the analysis of evolution is to determine which methods are effective for detecting differences between strains. Using a Bio Rad imager and pulse field gel electrophoresis, veterinary medical officer Jean Guard-Bouldin (left) and technicians Carolina Hall (center) and Cheryl Pearson-Gresham classify strains that are being used to produce mutational maps.

“This information about differences between genomes could help streamline the process of finding out how human disease organisms evolve to become more virulent,” says Guard-Bouldin. “The main focus for us now is to continue sequencing entire genomes and searching for more genetic changes that help us understand the *Salmonella* organism.

“Up until recently, genomic techniques for delving this deeply into the genetic code of multiple *Salmonella* strains weren't available or cost effective.

“If we can understand how *Salmonella* evolved to become pathogenic, perhaps we can apply the same principles to other foodborne pathogens and begin to study foodborne illness the way influenza is being monitored—with equal emphasis on the importance of small, as well as large, genetic changes.”

According to the Centers for Disease Control and Prevention, about 40,000 cases of salmonellosis are reported in the United States every year. Most result in diarrhea, fever, and abdominal cramps lasting 4 to 7 days, but severe cases caused about 70 deaths in 2000. Adequate cooking eliminates the risk of infection from eggs.—By **Sharon Durham**, ARS.

This research is part of Food Safety, an ARS national program (#108) described on the World Wide Web at www.nps.ars.usda.gov.

Jean Guard-Bouldin is in the USDA-ARS Egg Safety and Quality Research Unit, Richard B. Russell Research Center, 950 College Station Rd., Athens, GA 30605; phone (706) 546-3446, fax (706) 546-3035, e-mail jean.guard.bouldin@ars.usda.gov. ✪