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Salmonella enteritidis Pilot Project

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Salmonella enteritidis has long been a vexing problem for the food industry as these bacteria multiply at a great rate in improperly stored foods or on improperly cleaned utensils or surfaces. One infected egg, for example, in a batch of scrambled eggs left on a steamtable for a few hours, can contaminate the entire dish. In most people, *S. enteritidis* can cause stomach and intestinal upsets, though the illness can be very serious in young children and the elderly; in rare instances it results in death.

The *S. enteritidis* organism can be found in the intestinal tract of humans, domestic and wild animals, especially rodents, and presents a problem to the poultry industry because it is found in eggs.

The Pennsylvania poultry industry, with funding from the USDA and the Pennsylvania Department of Agriculture, has mounted a pilot program to reduce the number of *S. enteritidis* infected eggs that reach the market. This voluntary program encompasses not only the testing of eggs but also testing of pullets, layers, their environment, and a study of the rodent population in chicken houses, as well as a review of rodent control measures.

Testing is conducted by four laboratories, the Cooperative Poultry Diagnostic Laboratory at the University of Pennsylvania, the Cooperative Poultry Diagnostic Laboratory at Penn State University, the Summerdale State-Federal Cooperative Laboratory at Summerdale, PA, and the National Veterinary Services Laboratory at Ames, IA.

Testing of eggs began in mid-July and so far 92,000 eggs from 51 houses have been tested. Eggs from seven flocks have been found to be contaminated with *S. enteritidis*. "We test eggs only if layers, trapped rodents, or the chicken house have tested positive for *S. enteritidis,*" explained Dr. Robert Eckroade, associate professor of pathology at Penn's New Bolton Center and director of the Cooperative Poultry Diagnostic Laboratory at the School. "Though a positive reading of any of these factors does not mean that the eggs are contaminated."

When eggs are tested, the farmer submits 1,000 eggs every other week for four tests. These eggs are broken and put in batches of 100 that are held at room temperature for two days. Then samples from these batches are cultured on agar plates for another two days. If *S. enteritidis* grows, that sample is sent to the National Veterinary Services Laboratory at Ames, IA for serotyping of the isolate. The test takes about two weeks from start to finish. If any of the eggs are found to be positive for *S. enteritidis* then all the eggs from that flock have to go to the breaker market; they are pasteurized to kill any bacteria. If the samples test negative four consecutive times, the eggs produced in that particular house are sold as graded whole shell eggs.

It is very difficult to eradicate *S. enteritidis* from the environment. "Mice and chickens go together," said Dr. Eckroade. "If a house is depopulated and then cleaned, the mice move out. But the moment chickens and feed are put back in, the mice return. They live in the walls and in the surrounding fields." Farmers try to control these pests through rodenticides, traps, and by keeping the area around the chicken house free of tall vegetation. But it is an ongoing battle. "I know of one farm that has used half a ton of rodenticide, and that did not eliminate all mice, but it did control them somewhat," Dr. Eckroade said. He explained that the mice do not appear to be sick even though they are carrying the *S. enteritidis* organism. "Chickens with *S. enteritidis* infection also rarely are sick, though the strain in Europe (phage type 4) is much more pathogenic than the strain seen here. There it often kills chickens."

Researchers at the three laboratories will be studying *S. enteritidis* in mice with particular emphasis on the infectious cycle. It has been discovered that the *S. enteritidis* strain is often identical in mice, chickens, eggs, and the environment in a given location.

Other factors being examined are farm biosecurity measures, cleaning and disinfection methods, and vaccination of chickens against *S. enteritidis*. Dr. Sherrill Davison, assistant professor of avian medicine and pathology, looked at the efficacy of disinfectants when mixed with water from various sources. Some farms use well water while others may use water from streams. Frequently there are other organisms or minerals present that may interfere with the action of the disinfectant. The study is still in progress, but Dr. Davison has found that chlorine is usually not an effective disinfectant for chicken houses.

She also is conducting vaccine studies in 12 flocks to determine whether the occurrence of *S. enteritidis* in eggs could be prevented by vaccination.

The *Salmonella enteritidis* pilot project study will take about two years and ultimately will involve 200 chicken houses. The researchers hope that their findings will lead to the development of a more effective *S. enteritidis* control protocol.

New, faster Salmonella enteritidis *Test*

The current tests used to identify *Salmonella enteritidis* take almost two weeks. Dr. Linda Keller, research associate professor at Penn's Veterinary School, has developed an ELISA (enzyme-linked immunosorbent assay) that can determine in just under 30 hours whether *Salmonella enteritidis* is present in a sample. "This test is based on a very specific monoclonal antibody to *Salmonella enteritidis*," said Dr. Keller. "We incubate the eggs for 24 hours at 37 C°. Then we take a sample of the material and "sandwich" it between the monoclonal antibodies. We can get a reading of the sample in about three hours."

Because of the large number of eggs being cultured at the Cooperative Poultry Diagnostic Laboratory at New Bolton Center, Dr. Keller has been able to compare the results of the ELISA test to those from the conventional test and verify the accuracy of the new test. "It's been very exciting and may be a commercial kit can be developed.

When a reading is negative in a suspect house, eggs can be diverted back faster to the grade market. This helps the farmer. And on the other side, if the results are positive, control measures can be instituted much quicker."

Herpes Virus as a Vector to Deliver Healthy Genes to Brain

Herpes virus, after an acute short infection, becomes latent in the nerve cells. This latency factor plays a key role in research by Dr. John Wolfe, assistant professor of pathology and medical genetics at the School, and Dr. Nigel Fraser of the Wistar Institute. Dr. Wolfe, a specialist in gene therapy, and Dr. Fraser, an expert on herpes virus, have collaborated to construct a herpes virus vector to deliver a healthy gene to central nervous system cells of mice afflicted with mucopolysaccharidosis (MPS) VII (Sly disease).

"The herpes virus is uniquely able to transfer genetic material to a brain cell without killing it because it is a latent virus," notes Dr. Fraser. "The advantage of the herpes virus is that it can transfer the gene directly to the target organ, without removing cells from the body and then reimplanting them," says Dr. Wolfe. "The healthy gene remains active in the brain cells, which is important for achieving permanent correction of the deficiency."

The researchers modified a herpes simplex virus (HSV-1) and inserted a normal gene for enzyme production near the location of the virus' latency gene to take advantage of that gene's ability to activate the normal gene. The reconstructed virus was injected into a group of mice with MPS VII and it was found that a small number of central nervous system cells then produced the proper enzyme over a prolonged period of time.

This is the first time that a genetic deficiency in diseased cells of the brain has been corrected by gene therapy. Drs. Wolfe and Fraser caution that further development is needed to correct enough cells to improve the disease in patients. It has been shown, in previous studies, that vector-transferred enzyme activity in MPS VII can result in correction of the metabolic defect. Thus the expression of vector-transferred gene in the central nervous system should correct the metabolic deficiency.

Sly disease, an inherited lysosomal storage disease, causes mental retardation and affects the liver, skeletal system, heart, eye and brain. It is a rare, but devestating fatal disease of humans, and has been identified in dogs and mice. It is estimated that 1 in 25,000 human births will result in some form of MPS. The disease is caused by a genetic deficiency where cells are unable to produce an enzyme needed to break down complex sugar molecules. The molecules accumulate in the cells, interfering with normal function.

A mouse afflicted with MPS VII.

Dr. Wolfe points out, that at this time, the number of cells showing activity of the corrected gene is small. However, as knowledge increases, the herpes virus vector could become an important vehicle to deliver corrective genes to the brain and other parts of the central nervous system.

The medical genetics group at the School has a long history of investigating the genetics, biochemistry, pathology, and treatments of animals with genetic diseases. Studies of these naturally-occurring counterparts of human genetic diseases have contributed to understanding both human and animal health. The Wistar Institute is an independent basic science research center located on the Penn campus with programs in virology, molecular genetics, cell and developmental biology, cancer therapies, structural biology and immunology.

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