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Innovative NGS workflow accelerates *Salmonella* detection and typing, aiding control

Biosecurity and processing interventions can reduce prevalence, but detection is what confirms whether those systems are working as intended.

A new rapid testing method is helping poultry producers detect *Salmonella* faster and more efficiently than ever before. The method, developed by Ceva Animal Health, dramatically shortens turnaround time while reducing testing costs by nearly two-thirds.

"This innovation gives us faster, more accurate insights into what's happening in poultry production," said James Mills, scientist at Ceva Animal Health. "That speed makes a real difference for surveillance programs and, ultimately, for food safety."

A persistent threat across the production chain

Although *Salmonella* rarely makes birds sick, it remains one of the most significant foodborne pathogens globally — responsible for more than 90 million human illness cases and about 155,000 deaths each year.[1] The bacterium can enter broiler production at multiple points, from breeder flocks and hatcheries to live operations and processing plants.

"*Salmonella* Enteritidis is especially known for vertical transmission through eggs," Mills explained. "Other serotypes often come from the environment — things like rodents, insects, wild birds or contaminated feed or water." He emphasized that pest control, including insects, plays a larger role in *Salmonella* introduction than many producers realize.

Because contamination can occur at so many points, successful control depends on prevention, vaccination and rigorous monitoring. Biosecurity and processing interventions can reduce prevalence, but detection is what

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confirms whether those systems are working as intended.

The need for faster, more precise detection

A strong *Salmonella*-control program starts with effective surveillance. Sampling key points such as hatchery chick papers, environmental boot swabs and carcass rinses provides critical insight into prevalence and serotype distribution.

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"The challenge is that traditional methods take too long," Mills said. "By the time you have results, conditions on the farm or in the plant may have changed."

The long-standing gold standard for identifying *Salmonella* — culture and biochemical testing under the Kaufmann-White classification — is labor-intensive, subjective and slow. With growing regulatory focus on pre-harvest monitoring, the industry needs faster, higher-throughput approaches that maintain or improve accuracy.

Mills explained that Ceva's drive to improve speed and accuracy was initially motivated by proposed USDA regulations that would have classified *Salmonella* as an adulterant in poultry products — a move that would have required pre-harvest testing on live birds.

"Even though those regulations didn't go forward, we took that challenge to heart," he said. "Producers still need faster ways to understand what's present before birds reach the plant."

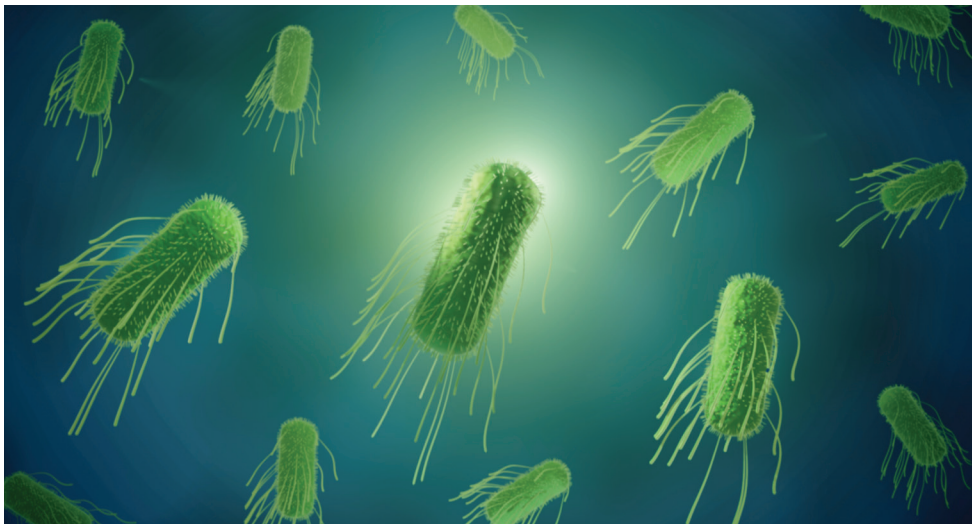
A new generation of *Salmonella* testing

Ceva's Scientific Support and Investigation Unit, working with the company's Next Generation Sequencing group, developed a molecular method that meets that challenge head-on. The approach combines intergenic sequence ribotyping (ISR) with next generation sequencing (NGS) to identify *Salmonella* serotypes directly from enriched samples, without requiring culture or isolation.

"In a single reaction, we can detect and identify more than 130 different *Salmonella* serotypes," Mills said. "That's a huge improvement in both efficiency and insight."

Mills noted that the method, which uses Oxford Nanopore sequencing, evolved from an earlier PCR-Sanger workflow. "Coupling ISR with NGS turned what used to take weeks into something we can do in just a few days," he said.

The ISR-NGS workflow has proven more sensitive than standard methods, detecting a greater number of positive samples and serotypes in carcass rinses and chick papers, and showing



equivalent accuracy with boot swabs. Transitioning to this system reduced the cost of goods by about 65% and shortened reporting from weeks to just a few days.

Faster results, smarter vaccine strategies

Detection is only one part of a comprehensive control program. Vaccination — using both commercial live and killed autogenous products — remains key to reducing *Salmonella* in poultry. But designing an effective autogenous vaccine depends on knowing which serotypes are circulating in the field.

"One of the first questions producers ask when preparing an autogenous vaccine is, 'Which strains should we use?'" Mills said. "Rapid, accurate identification gives us that answer."

The ISR-NGS assay enables labs to focus on priority serotypes such as Enteritidis, Typhimurium (biphasic and monophasic forms) and Infantis. Positive samples for these are cultured for isolation and subjected to whole-genome sequencing (WGS) to assess antimicrobial resistance, virulence factors and plasmid content.

Mills added that the method's sensitivity helps identify serotypes present at very low levels. "Sometimes we detect a serotype that's present in such low numbers we can't isolate it," he said. "But knowing it's present still helps us target our vaccine and biosecurity measures more effectively."

Further single nucleotide polymorphism and multi-locus sequence-typing analyses help track epidemiological relationships between isolates — revealing how *Salmonella* may move through the production chain or persist in certain environments.

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From innovation to industry impact

After vaccines are applied, continued surveillance ensures that control programs remain effective and that the right serotypes are being targeted over time, Mills said. By integrating ISR-NGS and WGS into the workflow, Ceva is giving producers a faster, more complete understanding of *Salmonella* dynamics in their systems.

"Speed and precision go hand in hand," Mills stressed. "When we can detect serotypes sooner and trace them more accurately, we can make smarter, faster decisions to protect both poultry and consumers."

He added that the streamlined workflow also frees up time for Ceva scientists to focus on deeper analysis and customer support. "We're not just grinding away in the lab producing data," he said. "Now we can analyze that data and provide meaningful insights to our customers."

Shorter turnaround times mean quicker feedback to production teams, more informed decisions about vaccination and hygiene programs, and more efficient use of resources, he added. For integrators managing multiple complexes or regions, the ability to monitor serotypes in near real-time also provides valuable benchmarking data.

"Broiler breeder vaccination and ongoing surveillance have already reduced key *Salmonella* serotypes in broiler production," Mills said. "Our new sequencing technology builds on that success — delivering actionable results faster and helping the industry stay ahead of an ever-present food-safety challenge."

[1] Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM. The Global Burden of Nontyphoidal Salmonella Gastroenteritis. Food Safety Clinic Infect Dis. 2010;50:882-889.

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