

Experimental vaccine offers improved protection for poultry

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Chickens are vulnerable to a range of infectious diseases similar to those found in humans. Fowl typhoid is a widespread and devastating illness, particularly in the developing world, where the birds are a vital source of income and nutrition.

Now, Ken Roland and his colleagues at the Biodesign Institute at Arizona State University have developed a candidate vaccine to safeguard poultry from fowl typhoid infection, while also providing protection from a related human [bacterial strain](#) – Salmonella Enteritidis.

"Fowl typhoid, caused by Salmonella Gallinarum, an avian-specific pathogen, accounts for about 10 percent mortality of chickens in the developing world, though this disease is often under-reported," Roland explains.

The group's clever approach to immunization relies on a modified strain of Salmonella Gallinarum that produces a robust immune response in Rhode Island Red chickens, similar to that produced by the naturally-occurring pathogen. Once a strong, system-wide immune response has been elicited however, a built-in mechanism disables the gene responsible for [bacterial virulence](#). The technique provides better protection from fowl typhoid compared with existing vaccines, while also offering an increased level of safety.

The group's research results recently appeared in the journal *Vaccine*.

Salmonella Gallinarum, causative agent of fowl typhoid, attacks birds of all ages, particularly broiler parents and brown-shell egg layers. While chickens are most commonly affected, the disease can also infect many other types of birds, including turkeys, game birds, bullfinches, guinea fowls, sparrows, parrots and canaries. Fowl typhoid is responsible for widespread morbidity and mortality in poultry, particularly in Africa, Asia, Europe and Latin America.

"In many developing countries, chickens represent far more than just a food source, although it is typically the primary source of [animal protein](#)," Roland says. "The free-range flock scenario exposes these birds to diseases carried by the wild bird population, which includes fowl typhoid. Increasing the quality and productivity of backyard chicken will thus provide an immediate impact on the quality of life of the rural poor."

Morbidity from fowl typhoid ranges from 10 percent to 100 percent in stressed or immunocompromised flocks. Birds typically acquire the infection through fecal-oral contamination or via the navel/yolk. The bacterium is fairly hearty, resistant to changes in climate and capable of surviving for months. Birds infected with *S. Gallinarum* typically display a variety of symptoms including lack of appetite, dejection, ruffled feathers, thirst, yellow diarrhea and a reluctance to move.

In attempting to combat such illnesses, various vaccine strategies have been developed. Live vaccines using weakened or attenuated *Salmonella* strains provide greater levels of protection than killed injectable vaccines by engaging all three branches of the immune defense, provoking humoral, mucosal and cell-mediated immunity, which is important for clearance of *Salmonella* infections.

Nevertheless, it remains a challenge for vaccinologists like Roland to create vaccines retaining strong immunogenicity once they have been

attenuated to ensure safety and reduce harmful reactions in the host. In the case of existing vaccines for fowl typhoid for example, full protection typically requires multiple injections, making it cost-prohibitive in much of the developing world. Further, the vaccine is virulent in some birds.

Roland and his colleagues have instead produced a single-dose oral vaccine. The experimental vaccine strains in the current study make use of a technique known as delayed attenuation, developed in the laboratory of Roy Curtiss, who directs the Institute's Center for Infectious Diseases and Vaccinology.

With delayed attenuation, the *Salmonella* strain enters the system with its native virulence intact, producing a strong, systemic immune response. Then, a key virulence-related gene switches off after a number of cell divisions, shutting down the bacterium's disease-causing potential.

The trick in delayed attenuation is to reengineer the *Salmonella* virulence gene so that it requires the artificial sugar arabinose for effective functioning. Once the bacterial cell's storehouse of arabinose is exhausted, the virulence gene essentially short-circuits and becomes inactive.

Three key virulence-related genes – *crp*, *rfaH* and *rfaH* – were previously identified from studies with the related pathogen *Salmonella typhimurium* in mice. Vaccine strains were constructed and tested in chickens with each of these genes subject to delayed attenuation via arabinose depletion. These strains were also compared for effectiveness with strains in which the virulence genes had been deleted altogether, rather than attenuated and with the wild-type form of the pathogen.

The best results in terms of immunogenicity and safety were produced by the vaccine strain bearing a *crp* gene subject to delayed attenuation.

The vaccine was avirulent and produced only minor internal lesions while offering superb protection from a lethal challenge of *S. Gallinarum*. Further, the presence of small quantities of arabinose in the birds' drinking water was not sufficient to disable the *crp* attenuation mechanism or affect virulence or immunogenicity.

The *crp* delayed attenuation strain was also tested for effectiveness against a lethal challenge of the human pathogen *Salmonella Enteritidis*. After four days, no detectable trace of *S. Enteritidis* appeared in systemic organs including the liver and spleen, indicating strong protection by the vaccine.

The researchers noted that the vaccine strain in which the *rfc* gene was deleted still exhibited full virulence in chickens, indicating that contrary to the mouse model of *Salmonella typhimurium*, *rfc* is a non-essential component for *S. Gallinarum* virulence in birds. In contrast, the *rfaH* deletion mutant was attenuated and protective, while the strain with arabinose-regulated *rfaH* expression retained full virulence.

The study indicates that delayed-attenuation *Salmonella* vaccines of the kind explored here can have wide applicability for the effective protection from a range of infectious diseases. In future efforts, the group hopes to fine-tune a vaccine strain with more than one attenuating mutation, at least one of which remains unaffected by dietary components, thereby offering improved safety along with maximum immunogenicity.

"Our goal," Roland says, "is to utilize 'high-tech' strategies to provide a 'low-tech,' easy-to-use, inexpensive vaccine, allowing everyone from backyard farmers to commercial hatcheries to vaccinate their flocks, resulting in better food security in the developing world."

Provided by Arizona State University

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