Genetically modified bacteria for state-of-the-art vaccines

Magic Rods

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Refined genetic modifications can turn menacing *Salmonella* cells into something precious for contemporary molecular biology: a new kind of vaccine that targets particular species of bacteria

Despite vast advances in the fields of immunology, microbiology, and molecular biology, infectious diseases remain a crucial medical and veterinary problem. Consumption of poultry meat contaminated with *Campylobacter* or *Salmonella* bacteria is the most frequent cause of human intestinal infections. As many as 19 species in the *Campylobacter* genus have been described, with human infections mainly being caused by *C. jejuni* and *C. coli*. The importance of such bacteria in causing human digestive infections has so far been underappreciated, mainly due to a lack of appropriate diagnostic methods. At present, *C. jejuni* is isolated from patients with symptoms of intestinal infections in the United States 3–4 times more frequently than *Salmonella* or *Shigella*! Unfortunately, research of the sort is not being carried out in Poland.

Poultry problems

Campylobacter infection can have dangerous consequences in humans. The symptoms vary, but mainly involve weakly to strongly infected states of the intestine, manifest in bloody and mucous diarrhea. In some individuals, however, it can lead to general bodily infection, with the contamination spreading to other organs and/or septicemia. Severe symptoms are mostly seen in individuals with reduced immunity (small children below 2 years of age, the elderly, post-cancer patients, and HIV carriers). *C. jejuni* infection also have the serious consequence of causing autoimmune diseases, such as reactive arthritis or neurological diseases: Guillain-Barré Syndrome (30–40% of cases result from a *Campylobacter* infection) or Miller-Fisher Syndrome.

The main source of such bacteria undoubtedly comes from chickens. They become colonized at an early stage of life, and the contamination spreads through farms very quickly, in practice affecting 100% of the birds in the flock. Interestingly, even though chicken intestines become colonized to a very high degree (as many as 10^{10} bacteria cells per gram of intestine content), this does not lead to symptoms of illness in them – a fact that prevents the culling of infected specimens. Things are entirely the opposite for humans: infection occurs relatively easily and a small quantity of *Campylobacter* rods will suffice. In developed countries, infection occurs most frequently through con-

Keith Weller/USDA

A new generation of vaccines is being developed thanks to refined genetic modifications. They can protect chickens from infection with *Campylobacter* bacteria, dangerous for humans

sumption of undercooked poultry, non-pasteurized milk, or water contaminated with the bacteria. Unfortunately, some 50%–90% of chicken carcasses offered for sale contain large quantities of bacteria from the *Campylobacter* genus.

Double weapon

Efforts to improve the level of hygiene at poultry farms have so far not produced the desired results in preventing *Campylobacter* contamination. Other methods of eliminating these rod-shaped bacteria now being tested in the US and EU envision the use of bacterial viruses or putting an anti-*Campylobacter* vaccine into widespread use for poultry and individuals in the higher-risk group.

Research now being carried out at Warsaw University's Departament of Bacterial Genetics aims to utilize specially developed *Salmonella* strains (*Salmonella enterica sv Typhimurium* χ3987) to produce a new generation of vaccines against *Campylobacter*. These genetically modified strains are not disease-causing, yet do evoke a strong immunological response. In developing such a vaccine against *Campylobacter* we are resorting to a kind of trick: introducing *Campylobacter* genes into the *Salmonella* strain, so that it produces not only its own proteins, but also *Campylobacter* proteins, which become recognized and remembered by the immune system. This strain can therefore act as an effective vaccine against both *Salmonella* and *Campylobacter*!

We have developed the prototype of such a "bivalent" vaccine. Our *Salmonella* has been designed to preclude it from reverting to its original pathological form, and to ensure that it is easily distinguishable from wild strains – something that is important in monitoring flocks. The *Salmonella enterica* χ3987 strain designed at the Departament of Bacterial Genetics was derived from χ3985, a chicken vaccine patented in the United States as far back as in 1999. Our prototype offers a great advantage: it can be delivered by being added to animal feed. Such feeding has proven to induce a high degree of production of specific antibodies (anti-*Salmonella* and anti-*Campylobacter*), of the IgG class in blood serum and of the s-IgA class in intestinal mucus. In order to boost what is called the humoral response, i.e. to increase the level of specific, secretory antibodies present in the intestinal lumen, we are currently testing the respective adjuvants. Of three *Campylobacter* antigens tested, the strongest immunogen has proved to be the product of the gene *cjaA*. A pilot "protection" experiment carried out in the laboratory has shown our prototype vaccine to be effective: only 3 of 25 immunized birds succumbed to *Campylobacter* infection, while all the chickens in the control group were colonized to a relatively high degree.

Future strategy

The proposed strategy of improving chicken immunity should effectively help prevent their digestive tracts from being colonized by two types of microorganisms that cause disease in humans, from the genus *Salmonella* and *Campylobacter*, and thereby significantly reduce the incidence of human infection. Moreover, the same vaccine could be used to immunize other species of poultry, such as turkeys, since *Campylobacter* infection is also a problem among their flocks as well. Such a vaccine will also help limit the rapid increase in the number of bacteria strains resistant to antibiotics. At the same time, immunizing chicken flocks against two microorganisms will simplify vaccine scheduling and be more economically efficient. *Salmonella* strains of weakened virulence are currently being tested in many laboratories for use as carriers of foreign genes from other organisms that cause disease in people or humans (viruses, bacterial, protozoans), and they may indeed come to form the basis for a new generation of vaccines. ■

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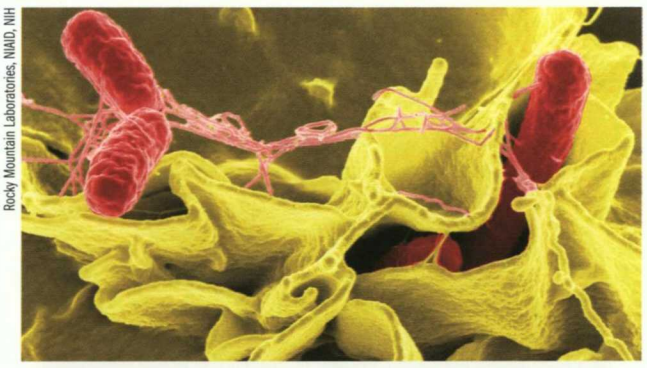

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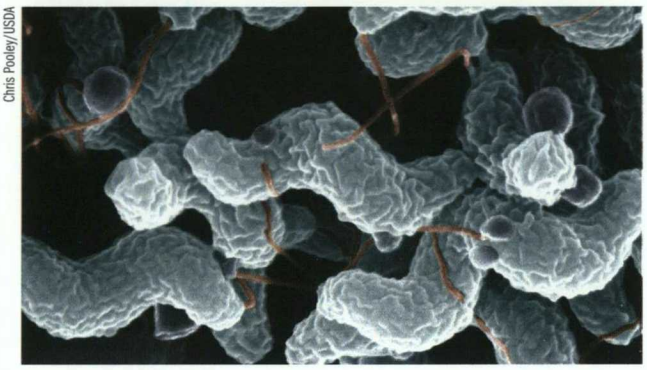

The nasty *Salmonella* (top) is rendered harmless by genetic modifications and can be used for developing modern vaccines. *Campylobacter jejuni* (bottom), neutral for chickens, is the main cause of serious infections of the human intestine

Further reading:

Wyszyńska A., Raczko A., Jagusztyn-Krynicka E.K. (2004). Oral immunization of chicken with avirulent *Salmonella* vaccine strain expressing *C. jejuni cjaA* gene elicits specific mucosal and systemic immune responses associated with protection against challenge with wild-type *Campylobacter*. *Vaccine*, 22, 1379–1386.

Ketley J., Konkel M.E. (2005). *Campylobacter: Molecular and Cellular Biology*. Washington: Horizon Bioscence.