

# Researchers improve recombinant attenuated salmonella vaccines

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Color-enhanced scanning electron micrograph showing *Salmonella typhimurium* (red) invading cultured human cells. Credit: Rocky Mountain Laboratories, NIAID, NIH

(Medical Xpress)—An innovative vaccine technology makes use of reengineered salmonella to deliver protective immunity. If such recombinant attenuated salmonella vaccines, or RASVs, can be perfected, they hold the promise of safe, low-cost, orally-administered defenses against viral, bacterial, fungal and parasitic infections.

In a new study, lead author Karen Brennenman and her colleagues at ASU's Biodesign Institute propose an improved method of screening salmonella vaccines in small animal studies and enhancing their effectiveness in humans.

The new research demonstrates a system for improving the ability of salmonella vaccine strains to survive the hostile environment of the stomach, where high acid concentrations are typically lethal for invasive bacteria. The data show a tenfold improvement in salmonella survivability in a [mouse model](#), modified to mimic stomach acid conditions in humans.

The research team – which included Crystal Willingham, Jacquelyn A. Kilbourne, Kenneth Roland and Roy Curtiss III (director of Biodesign's Center for Infectious Diseases and Vaccinology) – recently reported their results in the journal *PLOS ONE*.

"Over the years, the mouse model has taught us a lot about how Salmonella interacts with mammalian hosts. However, it turns out that the mouse model fails to provide relevant information on how Salmonella is able to deal with the extremely low pH of the human stomach," says Roland, corresponding author of the new study.

An RASV vaccine uses salmonella – causative agent of gastrointestinal disorders, including common food poisoning. Researchers in the Curtiss lab have been modifying salmonella by removing virulence factors that cause illness, and outfitting the bacterium with key antigens associated with the vaccine disease target.

Salmonella-based vaccines are capable of powerfully stimulating mucosal, humoral and cellular immunity against invasive pathogens, but to be effective, the vaccine cargo ships – the salmonella – must survive hostile conditions of the stomach.

While RASV results in mice have been encouraging, human trials have proven less favorable. The authors speculate that the high acid (low pH) conditions of human stomachs compared with mice may be partly responsible for the discrepancy.

Many types of salmonella exist, and the form affecting humans – *Salmonella enterica* serotype Typhi – is species-specific. To test RASV vaccines in vivo, prior to human clinical trials, requires the use of *Salmonella Typhimurium*, a closely related strain affecting mice and producing an illness similar to human typhoid.

One critical area in which mice differ from humans however, is in the acidity of their stomachs. For humans, the pH level in an empty stomach is between 1.0 and 3.0, whereas for mice, it is around 4.0.

"Finding a way to temporarily lower the stomach pH of a mouse sets the stage for further research into designing more effective oral vaccines, such as RASVs, and will provide new insights into how wild-type pathogenic bacteria survive this barrier. Our paper also demonstrates the utility of our previously described, novel acid-resistance systems to increase survival of RASVs in vivo at low pH," Roland says.

In the new study, high acid conditions in the mouse stomach were transiently induced through the injection of histamine. The histamine mouse model was capable of distinguishing *Salmonella* strains showing acid sensitivity or acid resistance, previously identified under laboratory conditions. The observed results in histamine mice were shown to closely correlate with in vitro acid resistance or sensitivity.

Low gastric pH is common to many host species, including humans, and acts in part as a defense mechanism to kill the majority of ingested microbes. The microbes, however, have actively developed strategies to attempt to survive such conditions, in a kind of evolutionary arms race.

Some microbes fare better than others under low pH, however. *S. Typhi* – the salmonella strain used for RASVs – is normally rather intolerant of low pH conditions, compared with the *S. Typhimurium* strains tested in mice.

A further factor of critical importance is the difference in gastric biology between mice in humans. Prior to RASV vaccine administration, both mice and humans are usually fasted in order to empty the upper part of the gastrointestinal tract. In mice, this has the effect of raising pH from around 3.0 to 4.0. In humans however, fasting pH is lowered, falling below 2.0. As the authors note, the mouse stomach after fasting poses only a mild challenge to the RASV invasive bacteria, whereas in the human stomach, conditions are hostile enough to kill most vaccine cells, undermining effectiveness.

Bacterial cells used for RASVs are generally not cultured for acid resistance, and are therefore particularly vulnerable to high acid gastric environments, further hampering their effectiveness. One common method for getting around the vulnerability of vaccine cells is to administer them in conjunction with an antacid or other agent designed to protect from low pH. The technique improves survivability – but at a cost. Without exposure to low pH, the [salmonella](#) vaccine bacteria lose valuable positional signals that help them colonize the intestine and successfully invade host tissue.

In the current study, heightened resistance to stomach acid was conferred to RASV strains through a special system using rhamnose-regulated arginine decarboxylase. The system was shown to dramatically increase survival of acid-sensitive *S. Typhi* in vitro at a pH of 2.5.

The researchers next sought to examine the effectiveness of RASVs containing the new acid resistance system in vivo, under the low pH conditions induced in the histamine mouse model. Results showed a

tenfold improvement in the ability of these modified strains to survive a gastric pH of 1.5 in the mouse stomach, and to successfully reach the intestinal tract. The implication is that such modified vaccine strains will likewise survive similar low pH conditions in humans, dramatically improving their effectiveness.

The study findings suggest that the histamine mouse model may be a useful tool for evaluating not only RASV candidates, but any orally administered microbial treatment (for example, pro-biotic bacteria). For microbes displaying acid sensitivity, the rhamnose-regulated arginine decarboxylase acid-resistance system can improve survivability under low pH and successful transit to the intestinal tract. Such improvement may elevate vaccine effectiveness while allowing a reduction in dose.

**More information:** Brenneman KE, Willingham C, Kilbourne JA, 3rd RC, Roland KL (2014) A Low Gastric pH Mouse Model to Evaluate Live Attenuated Bacterial Vaccines. *PLoS ONE* 9(1): e87411. [DOI: 10.1371/journal.pone.0087411](https://doi.org/10.1371/journal.pone.0087411)

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