Controlling Salmonella in the poultry gut: Diversity is key

When microorganisms that interact in a specific environment suppress the growth of pathogens, researchers call this ‘pathogen exclusion’. Salmonella is found in poultry intestines and can harm both the birds and humans. To control Salmonella growth, a balance between microorganisms that colonise the poultry gut is necessary. This involves complex interactions between microbes that we don’t yet fully understand. Dr Margie Lee at Virginia Tech in the USA has brought to light new and exciting information that takes our understanding of the gut microbiome a step further.

Competitive exclusion is the principle that two species competing for the same limited resources for survival cannot coexist in the same living environment or ‘ecological niche’. When one species has even the slightest advantage over another, it will use this to consume as many resources as possible, not leaving anything for the competing species, which will either become extinct or move to a new environment.

Pathogen exclusion is a type of competitive exclusion that refers to potentially harmful microorganisms (pathogens). Among other microorganism habitats, this phenomenon can take place inside animal intestines and affect the balance between friendly and potentially harmful microorganisms that make up the gut microbiome (the ecosystem of microbes in our gut). Competitive exclusion, besides the competition for limited nutrients, might also include competing for attachment sites on the inner lining of the bowel (mucosal) or the production of toxic substances by certain friendly bacteria that can restrict the growth of pathogens (antagonism). It is very likely, therefore, that pathogen exclusion involves a diverse community of intestinal microorganisms that work together to keep pathogen growth in remission.

**Chicken and Salmonella**

Salmonella is the name for one group of bacteria that cause gastroenteritis (food poisoning) in humans. One of the ways that the bacteria get transmitted to humans is by consumption of infected poultry meat or eggs. Salmonella bacteria often colonise the intestines of chickens and can be transmitted to humans via the consumption of infected meat or eggs.

**NEED FOR NEW TREATMENTS?**

It has previously been shown that day-of-hatch chicks that are exposed to the gut microbiome of healthy mature chickens develop a protective, diverse gut microbiome. This intervention makes them more resistant to Salmonella infection and colonisation, a phenomenon based on the principle of pathogen exclusion.

To become exposed to the mature chicken's microbiome, the chick has to receive a faecal transplant – which is the insertion of the mature chicken’s stomach bacteria into the chick’s gastrointestinal tract. Different products have been used so far to achieve this, including Aviguard, a competitive exclusion product derived from the intestinal contents of adult hens. These treatments have shown some efficiency in reducing Salmonella and other pathogens in chicks’ intestines. However, the lack of standardisation of the content of such products makes it difficult for them to be broadly used and distributed. This creates a need to develop novel products containing a single or multiple microbial species capable of excluding the pathogens.

Salmoneilla. She hopes to create a better understanding of how the gut microbiome imbalance can lead to colonisation by pathogens.

**A COMPLEX SYMBIOSIS**

Salmonella's ecological niche is the caecum, which is a sack at the end of the large bowel or colon. When present there, its numbers are usually 1 in 1,000 of the total microbes (gut microbiota). Salmonella is a scavenger bacterium that feeds on sugars produced by other gut bacteria called Clostridia. Clostridia are capable of breaking down bigger carbohydrate molecules present in the gut and therefore provide nutrients for other microbe species.

There is tough competition between bacterial species for these resources. Salmonella has an advantage over others, though, since it can metabolise end-products produced by Clostridia and other primary degraders using anaerobic respiration. This means it can break down molecules for energy without using oxygen. This interaction could potentially lead to the formation and maintenance of a friendly environment for both Clostridia and Salmonella.

Besides the cooperative interactions between certain species of the gut microbiome, there are also microbes that can interact with Salmonella unfavourably through competition, or antagonism. Some gut microbiota that compete with Salmonella for the same limited resources excrete by-products that limit the growth or kill Salmonella.
bacteria. This type of competitive exclusion is also the principle on which probiotics are developed.

**MEASURING PATHOGEN EXCLUSION**

Based on the above, Lee and her team conducted a study investigating the possible modes of competitive exclusion in the chicken gut, such as competition, antagonism, or attenuation of Salmonella. The researchers used two types of microbial communities for their experiment: the microorganisms of caecal contents from 35-day-old birds with high levels of Salmonella in their intestines (permissive community) and the microorganisms included in the commercial product Aviguard (exclusive community).

A medium made to imitate the caecal lumen’s nutritional environment was used to incubate the two types of culture with only added microbe communities along with the commercial product Aviguard strain did not grow as much in the absence of oxygen. It takes more than one or two species to directly control the growth of Salmonella in the gut of hens.

The results of the experiment were analysed and statistically processed to reveal that the Salmonella reporter strain did not grow as much in the presence of the exclusive community microbes compared to the permissive community ones. The microarray analysis also showed that the exclusive community significantly suppressed the expression of Salmonella’s host damaging genes (virulence genes), with 52 of them being silenced compared to 7 in the permissive community. Anti-inflammatory and anti-virulence molecules were also found at higher levels in bacteria of the exclusive community cultures, potentially secondary to the toxic action of microbial by-products such as butyrate or indole (mechanism of attenuation).

Further evidence that the exclusive community was producing antimicrobial substances was provided by the presence of certain enzymes produced by its bacteria which were at higher levels when the respective numbers of Salmonella bacteria were low in the chicken intestine.

Comparing the expression of bacterial genes in the permissive and exclusive communities to the control culture, it was found that over 75% of the 163 highly expressed genes of Salmonella grown with the permissive community were related to the bacteria’s ability to break down molecules for energy. This was true for only 17% of the highly expressed genes of Salmonella grown with the exclusive community. This suggests that circumstances within the permissive community were more favourable for Salmonella’s growth than inside the exclusive community. The exclusive community also affected Salmonella’s defence response. It could therefore make it susceptible to certain antimicrobial molecules produced by the chicken bowel cells that are toxic for Salmonella. Surprisingly, the competition with other species on nutrients did not affect Salmonella’s ability to utilise them – an outcome that reflects its metabolic versatility and its ability to break down a smorgasbord of energy molecules both in the presence and absence of oxygen.

**IS DIVERSITY THE KEY?**

In the case of Salmonella in chickens, competitive exclusion involves a combination of mechanisms, including competition, attenuation, and antagonism between member species of the gut microbiome. The findings of this study support the understanding that it takes more than one or two species to directly control the growth of Salmonella in the gut of hens. These chicken-friendly microbes collectively affect Salmonella’s metabolic versatility, reduce harmful pathogen activities, and produce toxic molecules to control the growth of pathogens such as Salmonella inside the gut.

**Detailed research**

To control Salmonella growth, a balance between microorganisms that colonise the poultry gut is necessary.

**Further evidence**

It takes more than one or two species to directly control the growth of Salmonella in the gut of hens.

**Research Objectives**

Dr. Margie Lee examines the effects of gut microbiome variety on pathogen spread in the poultry gut.

**Bio**

Dr. Margie Lee received her DVM from Virginia Tech in 1986 and PhD from the University of Georgia in 1990. Lee has over 90 publications focused on food safety, microbial virulence, antibiotic resistance, and molecular ecology of the chicken intestinal microbiome. Her earliest work describing the microbial succession of the chicken intestinal microbiome has over 1,000 citations.

**References**

Maurer, J.J., et al. (2024) Peeling back the many layers of competitive exclusion. Frontiers in Microbiology, 15. doi.org/10.3389/fmicb.2024.1342887


**Personal Response**

What inspired you to conduct this study?

Since graduate school, I’ve been fascinated by gut bacteria and why some are pathogens and some are not. And why the gut’s anatomical structure seems intended to farm the bacteria. It was clear that we were missing important concepts on pathogen control and a better understanding of the gut bacteria might reveal how nature does it.

What do the findings of your research say about the existing faecal transplant products and how could you improve them?

There are a few single organism probiotics that are effective in controlling intestinal pathogens, but these must be administered daily and may not improve the intestinal microbiome structure overall. Faecal transplant products consist of a community of bacteria that exhibit community behaviour in which diversity is a key characteristic. Now that we have a better understanding of the behaviours, we can better formulate these products.