**Engineering novel, safe, and effective vaccines against Avian Pathogenic *E. coli***

**Summary**

**Submitted to British Egg Marketing Board Education Trust for pump priming project “Engineering novel, safe, and effective vaccines against Avian Pathogenic *E. coli*”**

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Avian Colibacillosis refers to a range of syndromes caused by the bacterial species *Escherichia coli* and is one of the most common infectious bacterial diseases of the layer industry. Colibacillosis causes elevated morbidity and mortality leading to economic losses on a farm especially around the placement points, peak of egg production and throughout the late lay period. Vaccination against Colibacillosis has previously proved effective however, there is currently only one vaccine licenced for use.

This project sought to engineer a novel vaccine against avian pathogenic *E. coli* and combine the beneficial properties of probiotics with vaccines.

**2018-19**

This project is centred on a novel concept for the production of immunogenic antigens using a scalable protein expression system that has a long history of use in the food industry.

We aimed to generate hybrid proteins using an expression system in *L. lactis*, a food-grade bacterial species. These hybrid proteins will be anchored to the surface of probiotic strains in a co-culture system. Resulting probiotics can be used as vehicles to deliver these immunogenic antigens to mucosal surfaces of at-risk layer hens and broiler chickens.

Unfortunately, attempts at developing an expression system in *Lactococcus lactis* were unsuccessful. A novel expression system was developed in *E. coli*.

**2019-21**

The Flagella and Fimbriae in the bacterium *E. coli* are appendages that enable motility and attachment respectively. These appendages protrude from the bacteria and are involved in the interaction of *E. coli* with it’s environment and the host. These are often the first *E. coli* proteins that come in to contact with the avian host during infection. Considering they are surface exposed, they are also the first bacterial structures to be recognised by the avian host immune system.

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| **Figure 1:** A) Schematic representation of an *E. coli* bacterial cell. Flagella and Fimbriae are long appendage-like structures that protrude from the bacterial cell surface. Fimbriae are filamentous protein structures that often bind to receptors thereby facilitating colonisation of environments. Flagella are complicated structures made of repeating subunits which allow the bacteria to “swim” towards nutrients. These structures are surface-exposed and therefore commonly interact with the avian immune system. |

**Results**

Work in our lab at the University of Surrey School of Veterinary Medicine has identified the Flagella and Fimbrial sub-types exclusively associated with Avian Pathogenic *E. coli*. These proteins are ideal candidates for vaccine development. When the avian immune system recognises these proteins, it will induce an adaptive response that may protect avian hosts from subsequent infection.

We were able to express and detect the flagella subunit associated with avian pathogenic *E. coli* using a novel, expression plasmid designed specifically for this project. This plasmid can be adapted for the study of other avian pathogenic *E. coli* proteins of interest, including vaccine subunits and proteins that contribute to infection (virulence factors)

Probiotics are live bacterial organisms that are promoted as having health benefits. *E. coli* Nissle 1917 is a probiotic (named after the microbiologist, Alfred Nissle) that has been used in human medicine for over a century, primarily to treat Crohn’s disease and Ulcerative Colitis. This strain has also been shown to improve poultry gut health. The flagella associated with avian pathogenic *E. coli* was able to be expressed in this probiotic strain.

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| **Figure 2:** A summary of the approach used in this study to combine the beneficial properties of probiotics and vaccines. |

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*E. coli* Nissle 1917 expressing the flagella protein was compared to the original *E. coli* Nissle 1917 in its capability to elicit inflammatory response from HD11s, an avian macrophages cell line that can be reliably grown in laboratory conditions.

We determined that expression of the flagella protein evoked a slightly increased inflammatory response from avian immune cells, but had no impact on the ability of macrophages to kill the *E. coli* strains.

**Conclusion**

We have shown that probiotics and vaccines can be combined and engineered to combat avian colibacillosis and can feasibly be used to promote gut health and protect against disease.

We have also designed a system for the production and expression of proteins of interest from Avian Pathogenic *E. coli*. This system will be used in the coming years, to characterise proteins that are unique to certain sequence types of APEC. Notably, this system will be used in a BEMB-funded PhD project **“Control of avian pathogenic *Escherichia coli* by non-antibiotic compounds inducing trained immunity of chicken macrophages”.**