



VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

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1. TNA Provided

Name of the TNA project	Name of TNA user	Organisation of TNA user	Country of TNA user	Installation from the RI	Start date	End date	Number of units of access provided
1. Assessing the role of the acute phase protein and opsonin MBL in protective immune responses against erysipelas in chickens	Robert Söderlund	National Veterinary Institute, Sweden (SVA)	SE	AU-ANIS	2019-07-16	2019-08-24	5.95
2. The role of inverse autotransporter FdeC in Avian Pathogenic Escherichia coli adhesion	Rafal Kolenda	Wroclaw University of Environmental and Life Sciences	PL	AU-ANIS	2022-10-17	2022-12-09	4.43

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2. Final reports of each TNA provided

2.1 TNA 1

Assessing the role of the acute phase protein and opsonin MBL in protective immune responses against erysipelas in chickens.

Aim:

Erysipelas is an infectious disease that is a major problem in laying hens, especially in organic production. The disease is caused by the bacterium *Erysipelothrix rhusiopathiae* (ER), which can infect a wide range of species including humans. Basic knowledge on immune responses to ER in chickens has been lacking. However, we have monitored immune events during experimental infection of young conventional laying hen chickens and identified prominent responses of heterophils and the acute phase protein mannose-binding lectin (MBL) concurrent with bacteraemia during the early phases of infection. MBL has several functions in the immune system including acting as an opsonin for phagocytosis. Moreover, the ER capsule has mannose, a ligand for MBL, as a major component.

Thus, it is possible that the observed MBL responses in infected chickens play a role in the successful clearance of experimental ER infection observed in our studies. In contrast, the disease is often fatal under field conditions, making the identification of protective immune responses critical. At VetBioNet partner AU, there are two unique chicken lines (L10H and L10L) that have high and low MBL baseline levels and show high and low MBL responses upon microbial challenge. The aim of the current project was to try to elucidate the role of MBL in the chicken immune response to ER infection by performing an ER infection experiment at the AU facilities using these chicken lines.

Conclusion:

The most prominent finding from this experiment was the ER specific T-cell activation observed for spleen cells collected day 18 after infection. This is the first time ER specific T-cells have been studied in the chicken and gives novel information on the development of specific immunity to this infection.

Interestingly the responding T-cells included those of CD4+ phenotype, i.e. T-helper cells, as well as those of CD8b+ γ δ TCR- phenotype, i.e. CTL, which could indicate a TH1-type response. Such responses are generally considered important against intracellular pathogens and ER has indeed been described to cause intracellular infection, although this feature is often overlooked. These results are a foundation for further studies of T-cell responses to ER infection, which will be valuable for development of effective vaccines for chickens against erysipelas.

Due to the very varying clinical outcome of the current experimental ER infection results were regrettably not conclusive on the role of MBL in the chicken defence against ER infection. Among the chickens that did develop bacteraemia and clinical signs the trend was that more L10H chickens with bacteraemia survived compared to L10L chickens. This could indicate that high MBL levels played a role in controlling the infection in the presence of high numbers ER in the system. Moreover, in vitro T-cell responses were higher in magnitude for L10L chickens compared to those of L10H chickens, which could indicate that ER antigens persisted for longer in chickens with low MBL levels. However, due to the low number of birds with bacteraemia in each group these findings need to be further tested and confirmed.

The TNA study is published in "Veterinary Research". Watrang E, Sørensen Dalgaard T, Brødsgaard Kjaerup R, et al. *Erysipelothrix rhusiopathiae*-specific T-cell responses after

experimental infection of chickens selectively bred for high and low serum levels of mannose-binding lectin. *Vet Res.* 2022;53(1):105. Published 2022 Dec 12. doi:10.1186/s13567-022-01126-w

2.2 TNA 2

The role of inverse autotransporter FdeC in Avian Pathogenic Escherichia coli adhesion

Aim:

Avian Pathogenic Escherichia coli (APEC) is an extraintestinal pathotype with zoonotic potential and an etiological agent of colibacillosis. APEC infections are common in broiler chickens, laying hens, and turkeys, which leads to decreased weight gain or egg production. APEC is a cause of huge losses in the poultry industry worldwide. Successful establishment of APEC infection depends on the initial step – adhesion to host tissues. The expression of virulence factors during adhesion needs to be controlled to avoid exposition of immunogenic molecules and loss of energy due to production of unnecessary proteins. Dysregulation of virulence factors expression in *E. coli* might lead to decreased fitness during host colonization and as a result inability to cause disease. We observed that deletion of *fdeC* gene encoding for adhesin increases adhesion of APEC strain IMT5155 to chicken epithelial cells in vitro. Proteomics analysis indicated that deletion of *fdeC* increases inorganic ion transport that downregulates YbjN, which normally suppresses motility in WT strain. As our results from in vitro experiments show the importance of environmental factors on FdeC expression and adhesion of APEC to epithelial cells, we aimed to investigate the role of FdeC in APEC during infection of the host i.e. chicken. Therefore, we performed APEC chicken infection experiments at Aarhus University. As deletion of *fdeC* leads to higher expression of flagella, which might influence the recognition of APEC by immune system, chicken immune response to APEC infection was analysed at UEDIN/Roslin Institute (please see separate deliverable report from partner Roslin Institute, D29.1).

Conclusion:

The experimental procedures allowed us to determine the role of FdeC in APEC lung infection and chicken immune response to APEC infection. We observed that *fdeC* deletion mutant is attenuated in its ability to cause immune response in chickens compared to wild-type strain. Our in vitro experiments revealed motility-related adhesion increase in cell culture model and chicken infections confirmed that deletion of *fdeC* causes attenuation of APEC as a trade-off effect between virulence potential and adhesion.