IN VITRO PATHOGENICITY AND CYTOKINE RELEASE ASSESSMENT OF AVIAN PATHOGENIC *ESCHERICHIA COLI* STRAINS ON THE BIOMIMETIC PORCINE UROTHELIUM MODEL

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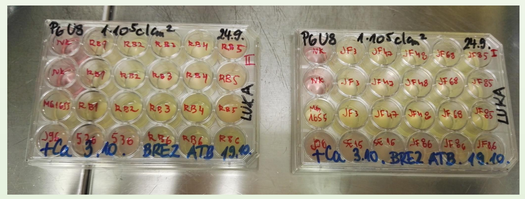
INTRODUCTION:

Avian pathogenic *Escherichia coli* (APEC), the *E. coli* associated with infections in wild and domestic birds (chickens, ducks, turkeys and geese), cause considerable financial losses in the poultry industry [1]. In addition, as APEC are closely related to human uropathogenic *E. coli* (UPEC), they also pose a serious risk to human health due to the possible transmission from birds to humans [1, 2, 3].



Picture: *in vitro* model after the infection with different *E. coli* strains

PURPOSE OF RESEARCH:

Our aim was to use the established biomimetic porcine urothelium model, a simple, fast, cost-effective and medically relevant model to study human UPEC pathogenicity [4], to evaluate APEC pathogenicity for humans. Furthermore, the total cytokine production in response to the infection of the model with APEC strains was investigated.

MATERIALS AND METHODS

Bacterial strains were firstly incubated in LB medium overnight and then diluted 1:100 into fresh LB medium and grown for additional 3h. Afterwards, bacterial suspensions were adjusted to 10^8 bacterial cells/ml using Nephelometer. Finally, bacterial suspensions were centrifuged and bacteria resuspended in suitable volume of NPU cell culture medium to meet the multiplicity of infection 1:10. Newly prepared suspensions were applied onto the *in vitro* model and incubated for 3h. Afterwards, the supernatant was collected, the NPU cells were washed and finally treated with a mixture of enzymes for braking down the cell culture and freeing individual NPU cells. Subsequently, the NPU cells were stained with trypane blue dye and examined with the inverted light microscope. **The viability of the NPU cells** was determined as ratio of live NPU cells to total number of NPU cells in blank control based on the colour difference. Finally, based on the determined precentage of viability of NPU cells in response to infection with *E. coli* strains all tested natural strains were classified into one of the following groups: **Commensal group I (>75% viability)**, **Low pathogenic group II (74% – 65% viability)** and **Highly pathogenic group III (64% – 0% viability)**.

The biomimetic normal porcine urothelium (NPU) model

Natural avian *E. coli* strains

6 APEC strains
6 commensal fecal strains (AFEC)

Set of control *E. coli* strains

J96 and 536 - human uropathogenic strains
MG1655 - laboratory strain
SE15 - natural human commensal strain

To assess the **cytokine production of the NPU cells in response to infection with *E. coli* strains**, the amounts of cytokines released into the cell culture supernatant were determined with the commercially available Porcine ProcartaPlex™ Panel 1 kit for the simultaneous detection of **nine different cytokines (IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, IFN-α, IFN-γ and TNF-α).** Imediately after the 3h incubation period of the *in vitro* model with bacteria the cell culture supernatants were collected, centrifuged and filtered in order to prepare bacteria-free samples. The samples were fistly stored at −80 °C and later on prepared for the analysis according to the users' manual (5) of the kit and subsequently analysed using MagPix instrument (Lumiex, USA) for the simultaneous detection of multiple analytes. Based on the **fold change in total cytokine production of infected NPU cells** (in response to the infection of the model with *E. coli* strains) compared to the total cytokine production in the blank control sample the *E. coli* strains were classified into one of the following groups: **Low fold change group (from 1 to 1,99 fold change)**, **Moderate fold change group (from 2 to 2,99 fold change)** and **High fold change group (3 fold change and more)**.

RESULTS AND DISCUSSION

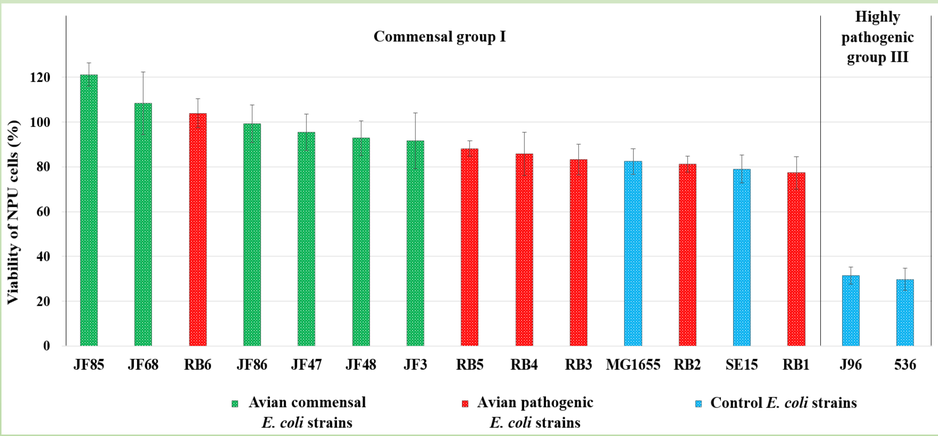


Figure 1: Viability of NPU cells after the infection of the *in vitro* model with different *E. coli* strains

According to the obtained results of the drop in the viability of NPU cells after the infection of the *in vitro* biomimetic porcine urothelium model with studied avian *E. coli* strains, **all tested *E. coli* strains (APEC and AFEC) were placed in the Commensal group I**. There were no avian *E. coli* strains classified as low pathogenic group II or highly pathogenic group III strains. The percentages of NPU cell viability obtained after the infection of the model are shown in **Fig. 1;** eventhough all avain *E. coli* strains are in the Commensal group I, 5 out of 6 **APEC** strains caused lower viability of NPU cells compared to **AFEC** strains. **These results indicate that the APEC strains tested had low potential to cause infections in humans.**

The analysis of the cytokine production of NPU cells in response to infection **Fig. 2** revealed differences in the amount of individual cytokines secreted as well as in total amount of cytokines secreted. **Different strains caused different cytokine production by infected NPU cells**. Since some commensal strains (JF48, JF47 and JF86) caused much higher total cytokine production compared to some **APEC** strains (RB5, RB2) **it is obvious that different strains have different strategies during infection and that pathogenic strains might have the ability to evade the immune response of the host's cells**.

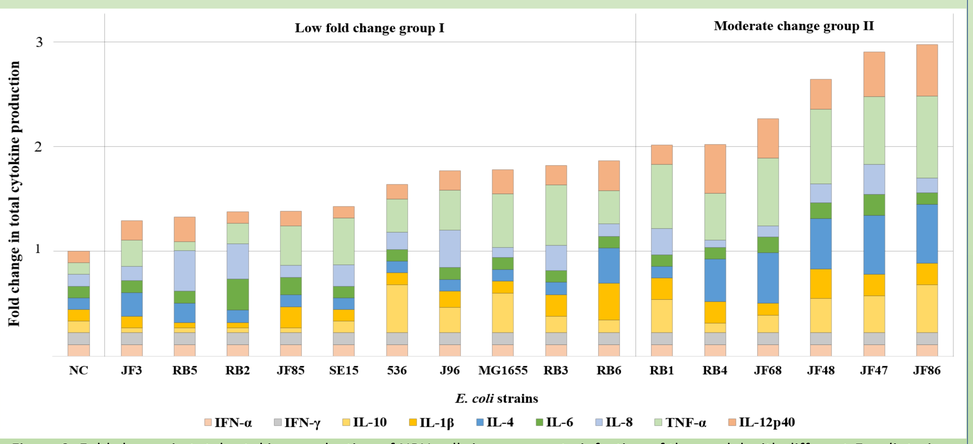


Figure 2: Fold change in total cytokine production of NPU cells in response to infection of the model with different *E. coli* strains

CONCLUSIONS:

The results obtained on our *in vitro* model revealed a **clear difference in viability of NPU cells**, when infected with different *E. coli* strains.

The analysis of the cytokine response revealed that the **NPU cells were able to respond to infection differently depending on the particular strain**, which contributes to the efficiency of the model.

Considering that NPU cells in cell culture retain their original morphological, molecular and ultrastructural characteristics and are genetically and physiologically similar to human urothelial cells, it can be assumed that the **results obtained with this model could be relevant for human medicine.** Hence, the established model is a **reliable model for assessing the potential zoonitic risk of APEC strains for humans**.

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ACKNOWLEDGEMENTS

This work was partially supported by the Slovenian Research Agency (P1-0198, P3-0108) and partially supported by the Government of Perm Krai (C-26/792).