A semi-automated *in vitro* model to study AMR-transfer dynamics in broiler chicken caecal microbial communities.

Ingrid Cardenas Rey[1,2] • Teresita Bello Gonzalez[1] • Kees Veldman[1] • Arjan de Visser[2] • Michael Brouwer[1]

1. Dept. of Bacteriology, Host Pathogen Interaction and Diagnostics Development, Wageningen Bioveterinary Research, Lelystad, The Netherlands • 2. Laboratory of Genetics, Wageningen University and Research, Wageningen, The Netherlands.

The continuous surveillance of antimicrobial resistance (AMR) in farm animals is critical to safeguarding public health. Alternative methods like *in vitro* gut models provide valuable insight and knowledge on the AMR transmission dynamics within and between microbial communities without using live animals. Understanding these microbial dynamics is essential for developing interventions that can reduce AMR spread among broilers and between broilers and humans. In this research, we aimed to: a) establish a semi-automated *in vitro* system able to mimic the physiological conditions of the broiler caecum (gastrointestinal organ) and maintain the main microbial communities, and b) study the dynamics of the *in vitro* cultured caecal microbiota over time.

Methods

Two continuous single-stage fermentation culture system (Applikon®) were employed to simulate the broiler caecal physiological conditions (pH, temperature, caecal movements and anaerobic environment) over time (Fig. 1).

Figure 1. Continuous single-stage fermentation culture system (Applikon®). **a.** Process control software (semi-automated workflow & real time monitoring) **b.** Advanced process controller (simulation of the broiler's caecal physiological conditions) **c.** Small-scale bioreactor (250 ml).

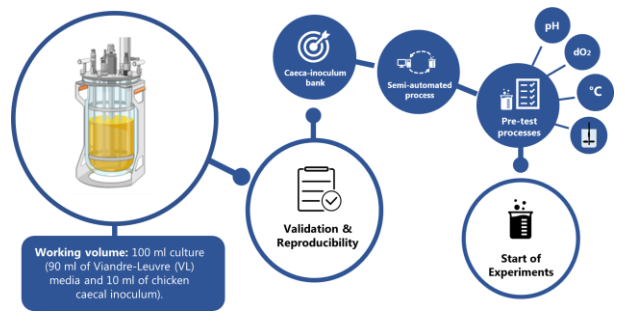

Figure 2. Broiler caecal *in vitro* system set up and optimisation. Validation and reproducibility steps performed before the start of the experiments.

A caecal inoculum bank and a semi-automated process workflow was developed to ensure reproducibility and reliability between processes. Pre-test processes were performed to evaluate and optimise sensors and controllers according to the broiler caecal physiological conditions (Fig. 2 and 3). Samples from both systems were daily collected for culture-independent (16S rRNA gene) analyses.

Results

All *in vitro* simulated caecal physiological conditions remained stable in both systems until the end of the processes (Fig. 4). Preliminary microbiota analyses showed (i) consistency of the bank inoculum and (ii) changes and stability in relative abundance of the phyla over time (Fig. 5). Additional microbial composition analyses (16S rRNA-gene analysis) are currently ongoing.

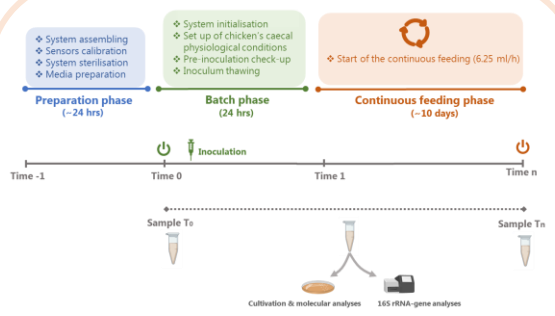

Figure 3. Semi automated-workflow. All experiments are performed in three phases (preparation, batch and continuous feeding phase). Batch and continuous phases are regulated by a feedback loop and real-time monitored until the end of the experiment.

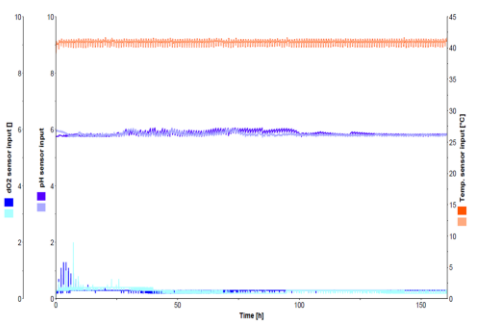

Figure 4. Real-time monitoring of process parameters. In this graph; pH, temperature, and anaerobic conditions (dO_2). Dark and light colours represent each *in vitro* system.

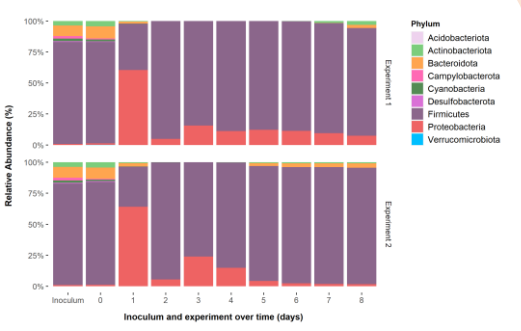

Figure 5. Relative abundance of Phyla observed in the inoculum and *in vitro* cultured microbiota over time from two independent experiments.

Our preliminary results demonstrate that a semi-automated *in vitro* broiler caecal system can stably reproduce the chicken's caecal physiological conditions and allow us to study the dynamics of the caecal microbial communities over time. Future experiments will study (i) Plasmid-transfer dynamics of Extended-Spectrum ß-Lactamases *E.coli* (ESBL-Ec) in the *in vitro* broiler caecal microbiome (ii) The effect of antibiotics and interventions (e.g. probiotics and prebiotics) on ESBL-Ec growth and plasmid transfer.

References

- Card, R. M. et al, mBio 2017 8:e00777-17.
- McDonald, J. A, Emerging Topics in Life Sciences (2017) 1 373–384
- Meimandipour, A et al, Letters in Applied Microbiology 49 (2009) 415–420.

Acknowledgements

Part of this work was funded by Dutch Ministry of Agriculture, Nature and Food Quality.

Contact: Ingrid.cardenasrey@wur.nl

This poster is part of the European Joint Programme One Health EJP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.