Final PhD Thesis Report

VIMOGUT

***In vitro* and in *vivo* analyses and MOdulation of the chicken GUT microbiota to combat AMR.**

Responsible OHEJP Partner: WBVR

GENERAL INFORMATION

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PhD Lead Supervisor	Mike Brouwer
PhD second supervisor	Arjan de Visser
Other supervisor (s)	Kees Veldman
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1. PhD Project team composition

PhD student: Ingrid Cardenas Rey
Wageningen University and Research. Wageningen, The Netherlands.
Wageningen Bioveterinary Research. Lelystad, The Netherlands.

Lead PhD supervisor: Mike Brouwer
Wageningen Bioveterinary Research. Lelystad, The Netherlands.

Second supervisor: Arjan de Visser
Wageningen University and Research. Wageningen, The Netherlands.

Other supervisors: Kees Veldman
Wageningen Bioveterinary Research. Lelystad, The Netherlands.

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2. Abstract

Antimicrobial resistance (AMR) tops the list of global health threats in humans and animals. Extended-spectrum beta-lactamases (ESBLs) are a relevant type of resistance mechanisms that inactivate a large group of critically important antibiotics and that have emerged globally in the livestock sector. Despite all the efforts in reducing antimicrobial consumption and banning growth promotors, a considerable proportion of ESBLs is still observed in the animal sector. AMR is a phenomenon that requires action and research using integrated strategies with a one-health approach. Based on this notion, the PhD project VIMOGUT brought together *in vivo* and *in vitro* studies to better understand the role of the chicken gut microbiota as an important ecological niche for the spread of AMR genes in the environment.

We have studied the successional dynamics of the caecal microbiota of developing broilers in relation to colonisation by ESBL- producing *Escherichia coli* (ESBL-Ec) in a commercial broiler farm. Our results suggested the presence of ESBL-*E. coli* is associated with mild but consistent reductions in broilers' caecal microbiota richness and transient microbiota compositional differences. We further documented the increasing prevalence and clonal spread of ESBL-*E. coli* in a single broilers' flock during a single production round and pointed at the farm environment as a likely source for ESBLs. We also advocated for additional research to more precisely understand whether the presence of ESBL-*E. coli* modulates the competitive landscape of the broiler microbiota or vice-versa. *In vitro* microbiota studies involving experimental ESBL-*E. coli* introduction could provide valuable answers to these questions, which can then be further confirmed under controlled animal experiments.

Another goal of the VIMOGUT PhD project was to contribute to more sustainable research by developing a cost-efficient and animal-friendly tool. We have developed an *in vitro* chicken caecal model capable of reproducing the physiological conditions required to culture the main members of the chicken gut microbiota. This milestone paved the road for future investigation on the effect of microbiota-targeted intervention studies and the horizontal transmission of AMR genes. In addition, the *in vitro* gut model also expands the possibilities of addressing other microbiota-AMR related research questions. Our results also show that optimisation steps of such tools are a continuous

process from which we learned that the choice of experimental settings impacts the *in vitro* cultured microbial community composition over time. Thus, pre-testing parameters and generating automated workflows are essential to reduce experimental bias, generate reliable data and draw meaningful conclusions.

The knowledge acquired during the VIMOGUT PhD project sheds light on the relationship between AMR bacteria and the broiler gut microbiota and highlights the need to consider the natural dynamics of host-microbiota development and the environment in the colonisation by resistant bacteria. Moreover, an *in vitro* model has been developed as a research tool to explore microbiota-based strategies to reduce the spread of AMR genes. This project supports the One Health approach to minimise the spread of AMR from animals to humans via the food chain in line with policies of the Dutch Ministry of Agriculture, Nature and Food Quality, as well as EFSA, ECDC, EMA, WOAH, WHO and FAO to tackle AMR.

3. Introduction and Objectives

The world is facing multiple health threats, and antimicrobial resistance (AMR) tops this list both in humans and animals (1). From all the resistance mechanisms, extended-spectrum beta-lactamases (ESBL) are specifically relevant due to their capacity to inactivate a large group of critically important antibiotics known as β-lactams (2). ESBLs have emerged globally in livestock and have been observed primarily in the *Enterobacteriaceae* family, especially in *Escherichia coli* strains (3). A high prevalence of ESBL commensal *E. coli* has been described in poultry production in the Netherlands during the last decade. Despite substantial efforts to reduce antimicrobial use in this sector, still a considerable proportion (23%) of Dutch broilers chicken tested positive for ESBL-*E. coli* in 2018 (4).

Previous studies suggest foodborne transmission of ESBLs from chicken meat to humans (5,6). However, the attribution of chickens or chicken meat as a source of ESBL *E. coli* to humans remains unclear (7). *E. coli* is a ubiquitous early coloniser of the chicken's gastrointestinal tract (GIT) and therefore it may play a crucial role as a potential reservoir of the ESBLs spread (8,9).

The chicken's GIT harbours complex and dynamic microbial communities better known as the microbiota. Microbiota members are present in very high numbers in the caeca, two blind pouches located between the small and large intestines where undigested food is fermented. These two pouches are a site of major water absorption and urea recycling. But most importantly, undigested food is retained in the caeca for up to 20 hrs (10). Those unique characteristics make the caeca a rich and favourable environment for bacteria to transfer multidrug resistance plasmids to each other (10).

The development of successful strategies to reduce the transfer of ESBL genes is only possible by understanding the successional dynamics of the chicken caecal microbiome and the role of ESBL *E. coli* therein (11). Although the successional dynamics of the caecal chicken microbiome have previously been studied (12,13), no studies accounted for the effect of ESBL-*E. coli* on the chicken caecal microbial communities, neither for the effect of *in vitro* microbiome interventions such as probiotics, prebiotics and synbiotics on ESBL transmission. Therefore, the aims of this PhD project are: i. to better understand the chicken caecal microbiota development in relation to ESBL-*E. coli* colonisation during the broiler

chicken lifespan and ii. to establish a chicken caecal *in vitro* model to assess the effect of ESBL *E. coli* colonisation, antibiotics, and microbiota-targeted on the horizontal transmission of ESBL plasmids.

The PhD project was divided into two components: *In vivo* and *in vitro* studies of the chicken caecal microbiota. The specific objectives of the *in vivo* were to identify key differences in the caecal microbial community composition of ESBL-colonised and ESBL non-colonised broiler chickens over time. Also, to detect any critical time window in the developing chicken caecal microbiota that enables the application of microbiota-targeted intervention (e.g. probiotics, prebiotics or feed additives) to prevent the spread of AMR genes.

On the other hand, the main objective of the *in vitro* component was to develop an *in vitro* caecal model that allows studying the effect of antibiotics and microbiota-targeted interventions on the horizontal AMR gene transfer dynamics in the *in vitro* chicken caecal microbiota.

Establishing a reproducible *in vitro* caecal model will pave the road for upcoming studies that aim to understand and reveal the interplay between the chicken caecal microbiota and AMR bacteria. Broiler meat is the most consumed animal protein worldwide. Therefore, more research is needed to understand the complex role of the caecal microbiota on broilers' health and well-being and consequently in food security. Moreover, the *in vitro* chicken caecal model is a step ahead in doing animal-friendly and sustainable research. Despite its limitations, this cost-efficient tool allows ample research with controlled parameters and real-time monitoring.

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4. Materials and Methods

In vivo* studies:*

- Succession in the caecal microbiota of developing broilers colonised by extended-spectrum β-lactamase-producing *Escherichia coli:* https://doi.org/10.1186/s42523-022-00199-4
- Development of the caecal microbiota of slow-growing broilers in relation to colonisation with ESBL-*E. coli*

  Currently ongoing:

  - Sample collection: caecal samples from slow growing broilers will be collected in a research center. Broilers will be followed during their production round (56 days). Broilers will be exposed to reused litter containing ESBL-*E. coli* and population colonisation dynamics will be screened overtime.
  - Samples will be used for ESBL-*E. coli* detection via selective culturing, ESBL gene detection and microbiota analysis.

***In vitro* studies:**

The objective of this component was to establish a reproducible *in vitro* model to approximate the physiological conditions of the chicken caeca to maintain its main microbial communities over time. A continuous single-stage fermentation culture system composed of a small-scale bioreactor (250ml, Applikon®), an advanced controller and process control software was employed.

Preparation of the chicken caeca inoculums

Sixty inoculums of 25 ml (10% suspension) were prepared and aliquoted in 60 ml glass bottles with rubber caps. Each 60 ml bottle contained 22.5 ml of PBS/glycerol 30% and 2.5 ml of caecal content. The caecal content was recovered from 46 random caeca obtained from a slaughterhouse and pooled in three groups. Each caecum was immersed in a beaker with ethanol 70% using tweezers and dried by tapping a paper towel. After disinfecting, the caecal content was recovered in a beaker by cutting an extreme of the caeca and squeezing it with the tweezers. The caecal content was mixed thoroughly with a spatula, and then 2.5 g was transferred to each bottle containing PBS/glycerol 30% and glass beads. Once weighed, the bottle was closed, mixed thoroughly, flushed with nitrogen for 2 minutes and stored at -80 °C. All caecal pools were tested for ESBL-*E. coli* to guarantee that the initial inoculum did not carry any ESBL-*E. coli*.

Validation and reproducibility of the *in vitro* model: Pre-test processes and a caecal microbiome survey were carried out to validate the chicken caecal *in vitro* model (Fig 1). Test processes included optimisation of the system to ensure pH control, anaerobic conditions by continuous monitoring of dissolved Oxygen percentage and temperature regulation. Another considered feature was the implementation of a pulsing stirrer profile which aimed to simulate the irregular and slow movements of the chicken caeca. Moreover, a semi-automated workflow was programmed allowing for real-time monitoring to ensure experimental reproducibility.

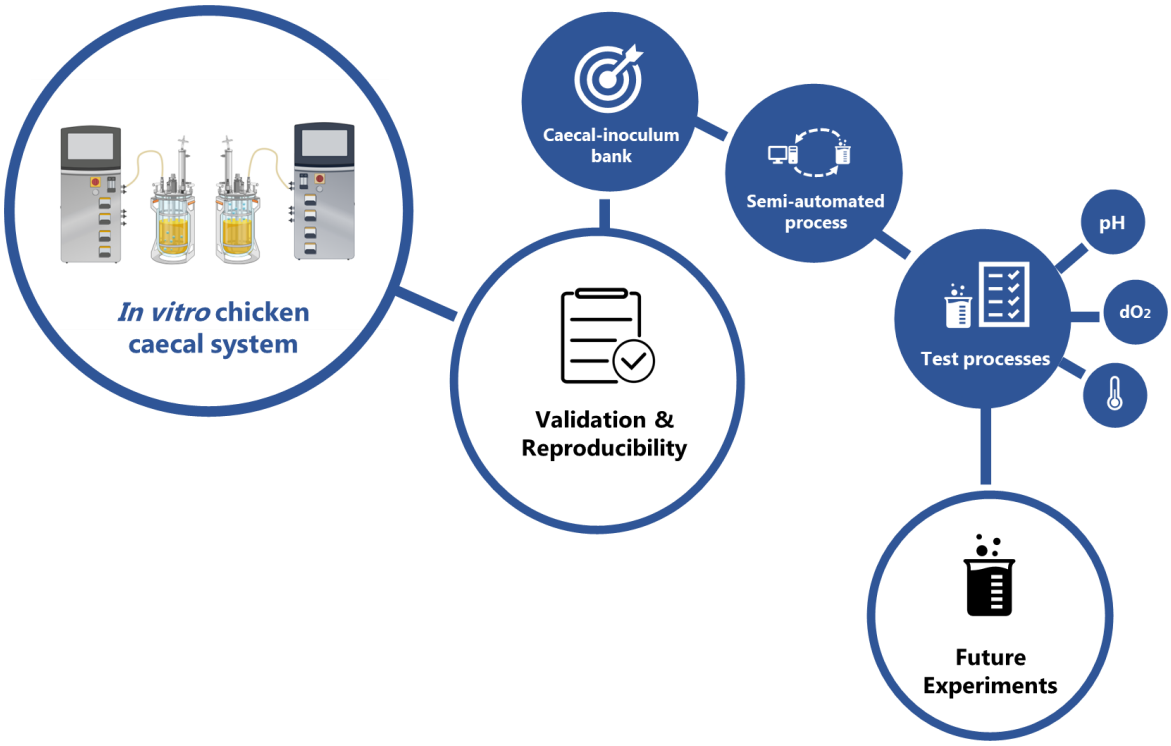

Fig 1. Steps followed to establish the *in vitro* chicken caecal model. The caecal inoculum bank and the semi-automated process were implemented to ensure reproducibility between experiments and reduce bias.

Experimental design:

Each *in vitro* test experiment included a treatment and a control bioreactor filled with 90 ml viande leuvre (VL) medium and 10 ml of caecal inoculum. The experiments were carried out in 3 phases: preparation, batch and continuous feeding (Fig 2). The preparation phase included media preparation, sensor calibration and system sterilisation. The batch phase was preceded by an initialisation step in which the optimal inoculation conditions were evaluated. Namely, a pH of 6.0, temperature of 41 °C, dissolved Oxygen <1% and a pulsing stirring profile (off/on 300 rpm).

The batch phase was implemented to provide a 24 hours adaptation period for the *in vitro* microbiota community. After the batch phase, each bioreactor was fed at a rate of 6.25 ml/h of LV medium. The inflow and outflow volume was equal during the whole experiment.

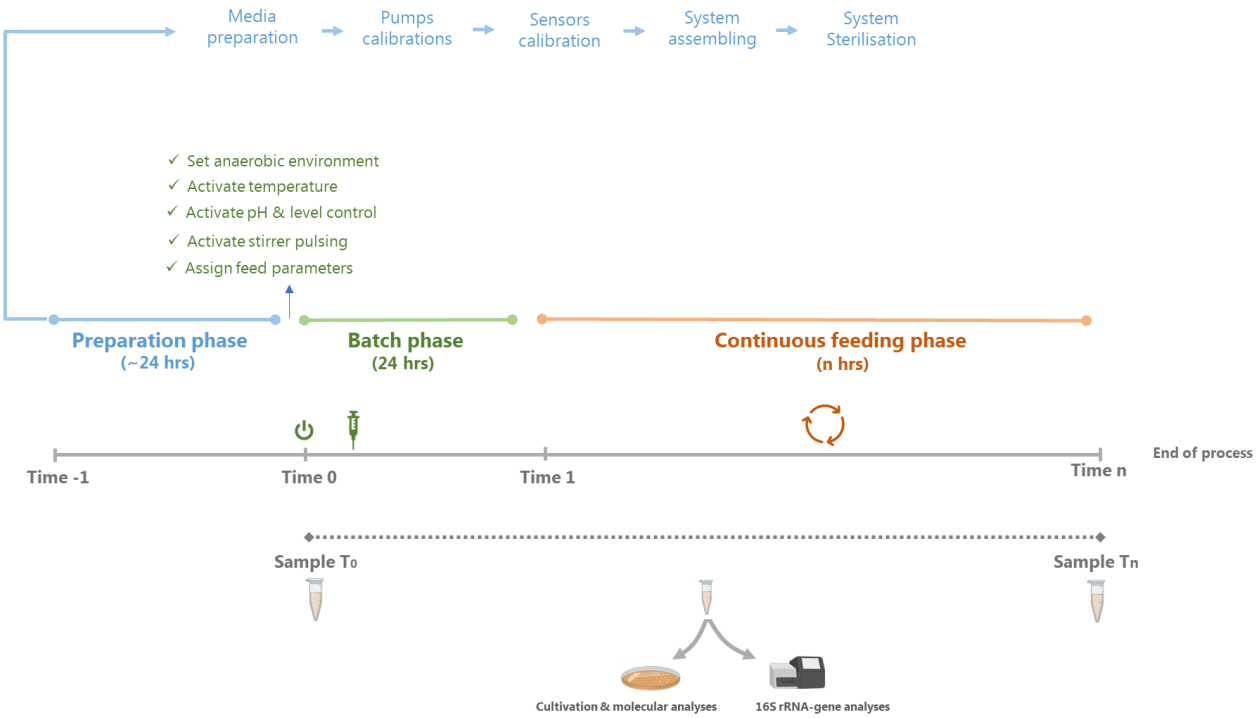

Fig 2. Representation of the different phases carried out in each experiment/test using the *in vitro* chicken caecal model.

Gassing strategies:

The choice of a gassing strategy is determinant to provide the optimal anaerobic environment for the viability of the caecal *in vitro* microbiota. Two gassing strategies were tested: i. continuous nitrogen flushing into the media (sparging method) and ii. Continuous nitrogen flushing in the bioreactor's overhead space (overlay method). Two bioreactors were run in parallel for 8 days, one with sparging and the other with the overlay method (Fig. 3). Continuous sparging adds nitrogen directly into the culture, creating a bubbling effect and providing more movement aside from the stirring. On the other hand, the overlay method adds nitrogen to the culture surface, generating no additional movements.

Caecal inoculum, sampling strategy and data analysis

Both bioreactors were inoculated with identical caecal inoculum as described above and operated with the same semi-automated workflow to simulate the physiological caecal conditions (pH of 6.0, 41 °C, <1% dissolved Oxygen and pulsing stirring profile). All the simulated conditions were real-time monitored during the whole experiment. Samples were taken at time 0 from the initial caecal inoculum (before and after being added to the bioreactor), and every 24 hours from each bioreactor until the end of the experiment and stored at -80 °C for further DNA isolation and microbiota analysis (V3-V4 region of the 16S rRNA gene). Raw data were filtered, trimmed, and chimaeras removed with the DADA2 package. Further data normalisation and microbiota downstream analyses were conducted in R 3.6.3 with the *phyloseq* v.1.30.0, *microbiome* v.2.1 and *vegan* (v.2.5-7) package.

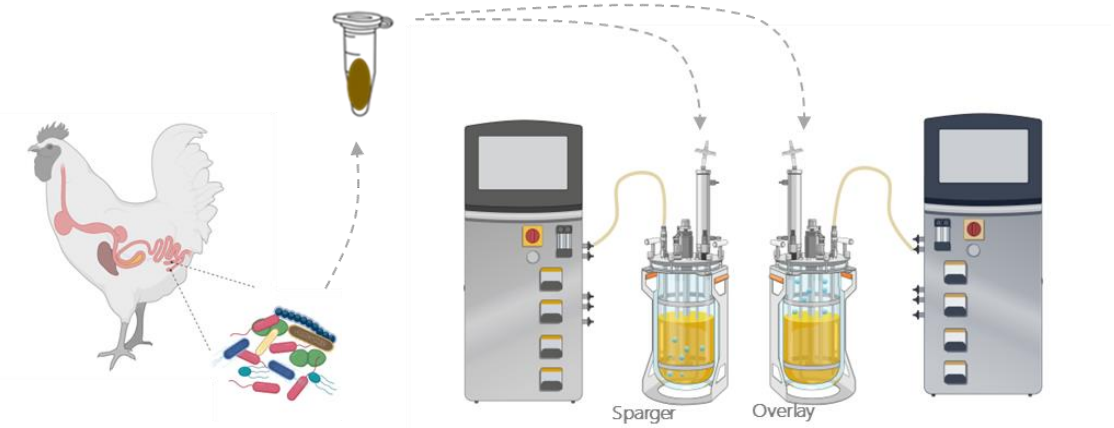

Fig 3. Schematic representation of the gassing strategies testing. The bioreactor on the left shows the nitrogen diffusion into the media, while the one in the right shows the nitrogen overhead flushing.

5. *Scientific Results and Discussion*

Chapter 1. Succession in the caecal microbiota of developing broilers colonised by extended-spectrum β-lactamase-producing *Escherichia coli*

In this chapter, we studied the successional dynamics of the caecal microbiota of developing broilers in a commercial flock during their production life cycle in relation to ESBL-*E. coli* (ESBL-Ec) colonisation. Broilers were categorised as ESBL-Ec colonised (ESBL-Ec+) or ESBL-Ec non-colonised (ESBL-Ec−) by selective bacterial culturing of caecal content. We compared the richness, evenness and composition of the caecal microbiota of both broilers' groups using 16S rRNA gene sequencing. We also assessed the combined role of age and ESBL-Ec status on the caecal microbiota development. We observed an increasing linear trend in the proportions of ESBL-Ec throughout the broilers' production round (Fig. 4A). Over time, the caecal microbial richness was consistently higher in ESBL-Ec− broilers, but significant differences between groups were found exclusively on day three (Fig. 4B). Bray–Curtis distance-based RDA (BC-dbRDA) analyses showed no explanatory power of ESBL-Ec status, while age explained 14% of the compositional variation of the caecal microbiota (Fig. 4C).

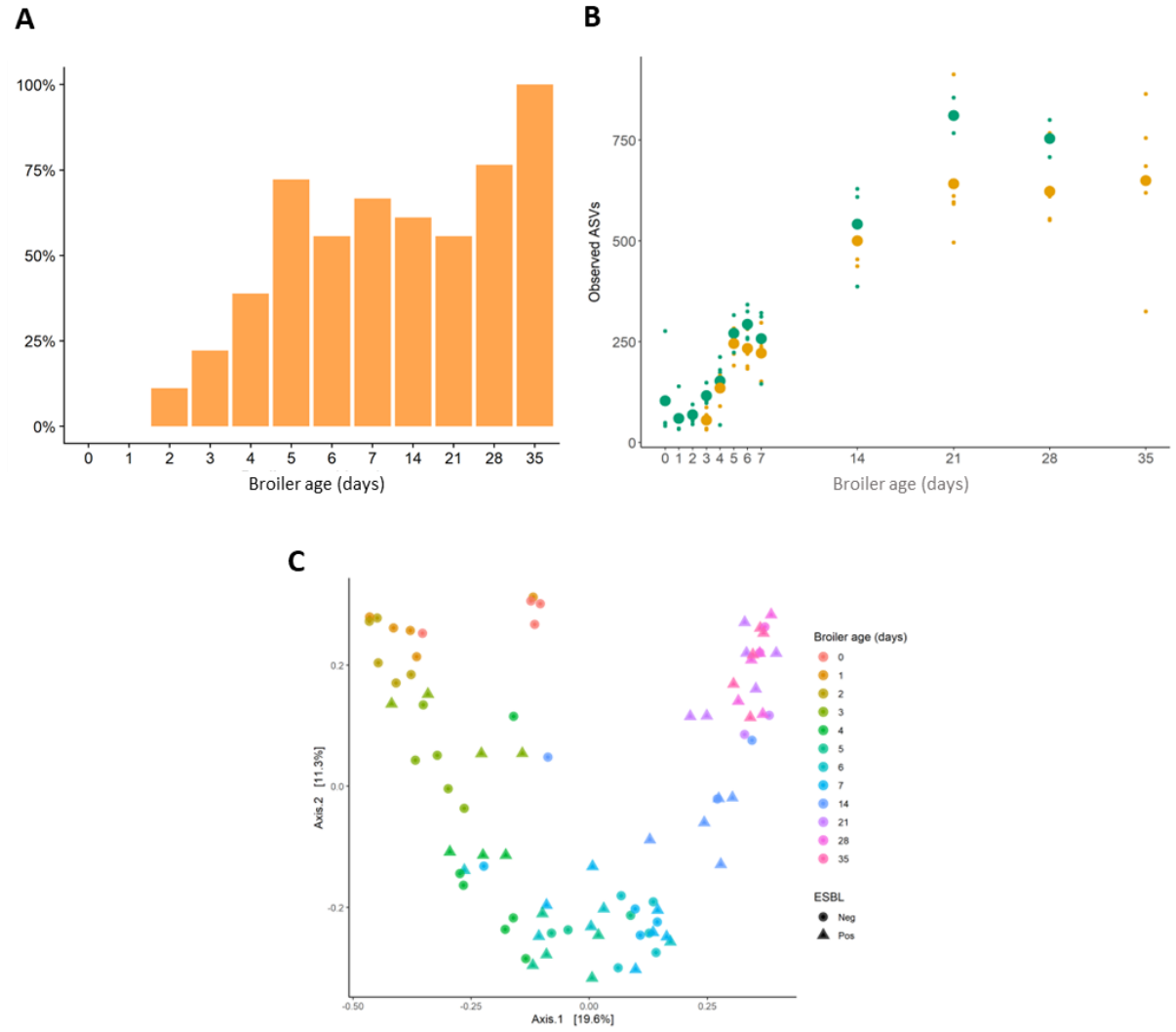

Fig. 4 A. Trends of ESBL-Ec prevalence in the total broiler population. **B.** Caecal microbiota richness and evenness of ESBL-Ec⁻ and ESBL-Ec⁺ broilers. Green dots represent ESBL-Ec- and orange the ESBL-Ec+ broilers. Small dots represent the amplicon sequence variants (ASVs) observed in each broiler. Enlarged dots represent the ASVs mean from all broiler samples at each time point. The asterisk denotes a $p < 0.05$ for the Wilcoxon rank-sum test. **C.** Changes in the caecal microbial community composition over time (days 0–35). Age explained 14% of the compositional variation of the caecal microbiome (BC-dbRDA: F (2,66) = 6.47, p = 0.001). ESBL status explained no variability.

Discussion:

Exploring the successional dynamics of the caecal microbiota of broilers can reveal windows of opportunity to implement intervention strategies that reduce the spread of resistant commensal and pathogenic bacteria. However, little is known about how changes in the developing caecal microbiota affect the prevalence of ESBL-Ec or how the presence of ESBL-Ec affects the microbiota. Here, we studied the caecal microbiota of developing commercial broilers in conventional farming conditions and classified them as ESBL-Ec+ and ESBL-Ec−. We found no clear divergence between these two

groups over time, suggesting that the presence of ESBL-Ec does not have consistent effects on the caecal microbiota of developing broilers.

All broilers tested negative for ESBL-Ec on days zero and one. ESBL-Ec was detected only from day two onwards, and its prevalence increased rapidly over time, suggesting that the farm environment was the likely source for ESBL-Ec colonisation. The AMR phenotype, ESBL-gene, plasmid typing and MLST results were identical for all samples, indicating a clonal spread of ESBL-Ec throughout the flock. The clonal distribution of ESBL-Ec has been associated with the high shedding of ESBL-Ec after colonisation in previous studies [14].

In contrast to the ESBL-Ec prevalence trends, the relative abundance of *Escherichia*/*Shigella* decreased over time in both groups of broilers. This reflects the broilers' microbiota dynamic development over time; as broilers age, the microbial diversity increases, causing shifts in bacterial abundance. Despite the continuous dominance of members of the phylum Firmicutes, Escherichia/Shigella persisted throughout the broilers' production life cycle. The observed changes in the caecal microbiota composition over time resembled those documented in previous studies: the relative abundance of Proteobacteria decreased as broilers aged, while the relative abundance of Firmicutes and Bacteroidetes gradually increased [16, 18, 19].

Consistent with the literature [16, 18, 19], the observed caecal microbial richness increased linearly as broilers aged in both groups. Overall, microbial richness was not significantly different between groups. However, a higher microbial richness was consistently observed in ESBL-Ec− compared to ESBL-Ec+ broilers over time. In line with this finding, ESBL-colonised broilers showed a much less diverse microbial composition on day three, with only five genera representing 86% of the total community. These results suggest small but consistent alterations in the microbiota's competitive landscape, which could be further explored with controlled, culture-based laboratory experiments.

As shown in previous research [16, 19, 23, 24], host ageing is one of the drivers of microbiota composition. In our study, age explained 14% of the microbial composition variability. The succession of the broilers' microbial communities was observed in three stages. The first stage (days 0–4) was dominated by Firmicutes and Proteobacteria, mainly by families Clostridiaceae, Enterococcaceae and Enterobacteriaceae. A decrease in Proteobacteria (< 10%) and a complete dominance (> 80%) by members of the Firmicutes (families Ruminococcaceae, Lachinospiraceae and Lactobacillaceae) characterised the second stage (days 5–14). In the third stage (days 21–35), Proteobacteria continued steadily decreasing (< 5%), while Bacteroidetes emerged, accounting for 16.6% of the total community. Firmicutes were still extensively represented principally by families Ruminococcaceae and Lachinospiraceae and the appearance of members of the Clostridiales. Despite the differences in study designs, these observations support previous results [16, 19], in which successional dynamics were also characterised in 3 stages and represented by similar phyla and families. On the other hand, the colonisation by ESBL-Ec did not explain any variability in the caecal microbiota composition in this study. Our results match previous observations [25], in which asymptomatic gut carriage of ESBL-Ec was not associated with differences in microbiota composition in humans.

To the best of our knowledge, this is the first study assessing the differences in the microbiota composition and diversity of ESBL-Ec+ and ESBL-Ec− broilers from a commercial farm throughout the production round. A previous experimental study examined the microbiota of broilers colonised with ESBL-Ec and treated with competitive exclusion products on days 5 and 21 [26]. However, the study

aimed to evaluate the effect of compartmentalisation and interventions on the transmission and prevention of ESBL-Ec colonisation in the broiler microbiota composition more than the effect of ESBL-Ec on the broilers caecal microbiota. Longitudinal-experimental studies which manipulate ESBL-Ec prevalence could reveal the process of colonisation of ESBL-Ec in the caecal microbiota and shed light on the relationship between AMR bacteria, the caecal microbiota, and potential avenues for microbiota-based control of AMR. Our study highlights the need to consider the natural dynamics of host-microbiota development and colonisation by resistant bacteria.

Chapter 2. Establishment of a semi-automated *in vitro* model to simulate the chicken caecal physiological conditions and maintain the main caecal microbiota communities.

This chapter describes the results of the set-up and optimisation of an *in vitro* chicken caecal model (Fig. 5). Several test runs were carried out to evaluate the performance of the semi-automated workflow. For each run there was a particular focus on pH control behaviour and culture agitation by the pulsing stirring response. Further testing was performed to learn about the best gassing strategy to maintain the optimal anaerobic conditions for the maintenance of the *in vitro* cultured chicken caecal microbiota.

Figure 5. Set up of *in vitro* chicken caecal system. The system includes two 250 ml bioreactors (control and treatment) operated in parallel with a semi-automated workflow with all the parameters needed to simulate some physiological conditions of the chicken caeca. Target parameters are controlled by two computers and dedicated software.

Effect of nitrogen gassing strategy on the *in vitro* cultured chicken caecal microbiota

All simulated physiological caecal conditions (dissolved Oxygen, pulsing stirring and temperature) except pH remained stable over time (Fig. 6). The pH fluctuations were associated with microbial activity during the continuous feeding phase.

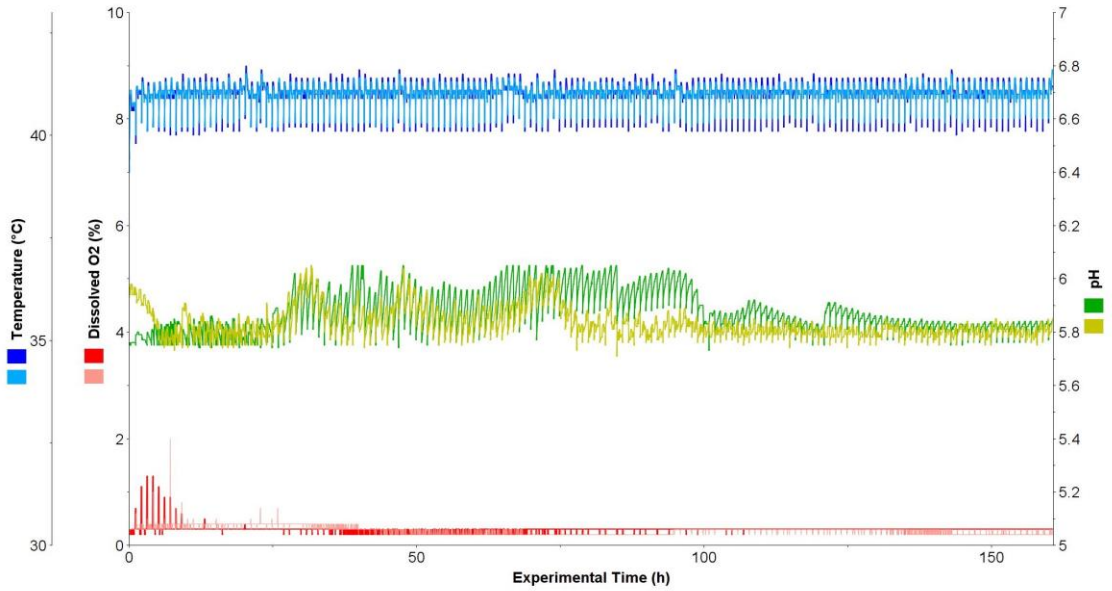

Figure 6. Real-time monitored parameters (pH, temperature, dissolved Oxygen). Parameters from the overlay and sparger methods are shown in light and dark colour, respectively.

Microbiota analysis

Bacterial abundance: Regardless of the gassing strategy and experiment time, *Firmicutes* was the most abundant phyla observed, making more than 90% of the *in vitro* bacterial community. *Bacteroidota, proteobacteria,* and *Actinobacteria* were observed in less proportion but consistently until the end of the experiment (Table 1).

Table 1. Relative abundance of the most dominant *in vitro* bacterial phyla detected in bioreactors aereated by the overlay and sparger method.

Experiment day	Overlay method	Sparger method
T1	*Firmicutes* (91,7%), *Bacteroidota* (3,4%), *Proteobacteria* (2,1%)	*Firmicutes* (92,7%), *Bacteroidota* (2,5%), *Proteobacteria* (2,3%)
T4	*Firmicutes* (94,7%), *Bacteroidota* (3,8%), *Proteobacteria* (0,78%)	*Firmicutes* (90,8%), *Proteobacteria* (8%), *Bacteroidota* (1,1%)
T7	*Firmicutes* (91,6%), *Bacteroidota* (5,8%), *Actinobacteriota* (2,8%)	*Firmicutes* (90%), *Bacteroidota* (6,6%), *Proteobacteria* (2,3%)

Differentially abundant taxa were observed only at the family and genus levels (table 2). The *In vitro* bacterial community from the sparger method showed a significant higher abundance of the genera *Butyricicoccus, Lachnospiraceae, Clostridioides*, and *Phyllobacterium* (Fig. 7A ; ANCOM-BC, $p<0.001$).

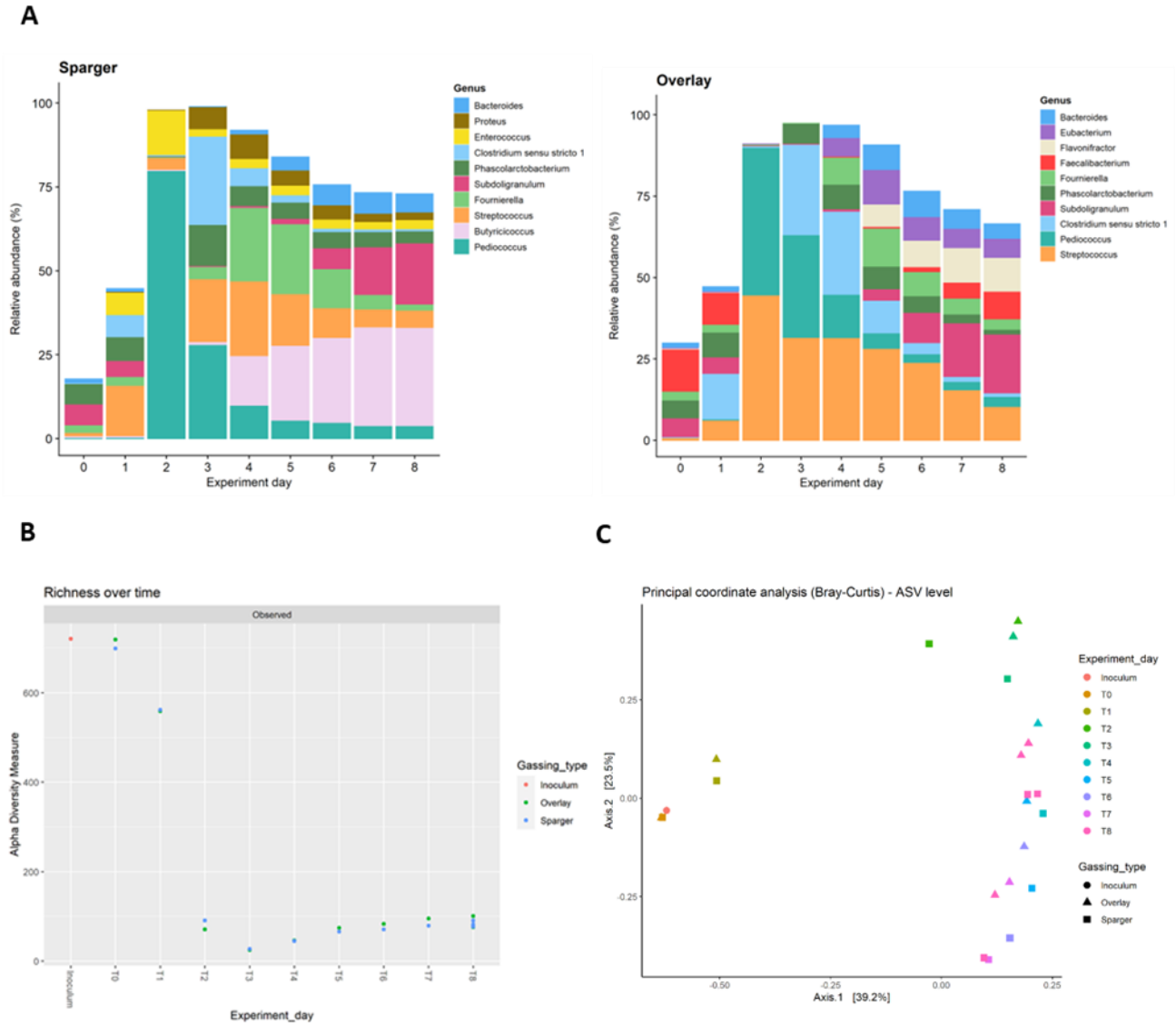

Figure 7. Alpha and beta diversity analyses of the *in vitro* chicken caecal microbiota in both bioreactors (overlay and sparger method). **A.** Comparison of the top ten genera' relative abundance over time. **B.** Observed microbial richness over time (ASV; Amplicon sequence variant) **C.** Principal coordinate analysis showing changes in the microbial community composition over time.

Table 2. Differentially abundant taxa among overlay and sparger method (ANCOM-BC, p<0,001).

Taxonomic level	Overlay method	Sparger method
Phylum	-	-
Family	-	*Rhizobiaceae, Butyricicoccaceae*
Genus	-	*Lachnospiraceae* AC2044 group, *Clostridioides, Phyllobacterium, Butyricicoccus*

Alpha diversity: Microbial richness fluctuated daily in both bioreactors (Fig. 7B). 721 amplicon sequence variants (ASVs) were detected in the initial caecal inoculum. During the batch phase, a decrease in ASVs was identically observed in both bioreactors (overlay 559 ASVs; sparger 562 ASVs). Microbial richness dropped markedly after the beginning of the continuous feeding. Still, it steadily increased until the last experimental day with 101 and 91 ASVs for Overlay and sparger, respectively. No significant differences were found in microbial richness between sparger and overlay (Kolmogorov-Smirnov test, p=0.833). We suggest that the low detection of ASVs on day 3 was associated with the beginning of the continuous feeding phase. A hypothesis is that the continuous removal of waste media culture after each feeding might select for fast-growing bacteria compared to slow-growing bacteria. Another possible reason for this result could be sampling and detection bias which calls for optimisation of methods for culture collection, including sampling time.

Beta diversity: *In vitro* microbiota community composition changes were determined using Bray-Curtis principal coordinate (BC-PCoA) and Adonis Permutation analyses. Changes in microbiota composition were associated mainly with experiment time (Adonis, $p < 0,001$) than with the gassing strategy. These results can also be correlated with the substantial decrease in microbial richness on days 2 to 8, depicted by clustering samples from the same experimental days (Fig. 7C).

6. Conclusions and Future work and perspectives

***In vivo* component:**

The first study from the *in vivo* component assessed the role of ESBL-*E. coli* colonisation in the successional dynamics of the caecal microbiota in developing broilers. Our results suggested the presence of ESBL-*E. coli* is associated with mild but consistent reduction in broilers' caecal microbiota richness and transient microbiota compositional differences. We further documented the prevalence trend and clonal spread of ESBL-*E. coli* in a single broilers' flock during a single production round and pointed at the farm environment as a likely source for ESBLs. Additional research is needed to more precisely understand whether the presence of ESBL-*E. coli* modulates the competitive landscape of the broiler microbiota or vice-versa. Such research questions can only be answered by controlled animal experiments where broilers or by *in vitro* microbiota- ESBL-*E. coli* colonisation experiments.

Current studies and future work

Current studies are being carried out to determine whether similar patterns in the successional caecal microbiota dynamics are observed in slow-growing broilers naturally exposed to ESBL-*E. coli*

colonisation. Moreover, data from a longitudinal study (OH-EJP ARDIG) on ESBL/AmpC plasmid epidemiology on Dutch broiler farms using hybrid sequencing techniques is currently being analysed. This work is also expected to be published in form of a research article.

***In vitro* component:**

We have developed an *in vitro* chicken caecal model capable of reproducing the physiological conditions required to culture the main microbiota members of the chicken caecal microbiota. The ability to culture a diverse bacterial community is relevant to study the effect of microbiota-targeted intervention studies on the horizontal transmission of AMR genes. Nevertheless, our results also show that optimisation steps are a continuous process from which we learn that the choice of experimental settings impacts the *in vitro* cultured microbial community composition over time. Thus, pre-testing parameters and generating automated workflows is essential to reduce experimental bias, generate reliable data and draw meaningful conclusions.

Current studies are focused on transferring the acquired expertise into a new *in vitro* system (DASbox mini bioreactor system, Eppendorf) which enables parallel operation of four bioreactors and comprises a more powerful and dedicated module for anaerobic experiments (Fig. 8). Despite the preliminary knowledge acquired from the study on the effect of nitrogen gassing strategy on the *in vitro* cultured chicken caecal microbiota, we believe no sharp conclusions can be drawn yet regarding of the best method (e.g. Overlay vs Sparging). Additional tests will be performed to confirm the results observed in this study. Such tests are planned using the parallel operation and dedicated anaerobic module of the newly acquired DASbox system mentioned above.

Future work in the new *in vitro* system work will evaluate i. the effect of media components on the maintenance of the *in vitro* caecal microbiota and ii. the effect of antibiotics and inoculation of ESBL-*E. coli* on the *in vitro* microbiota composition.

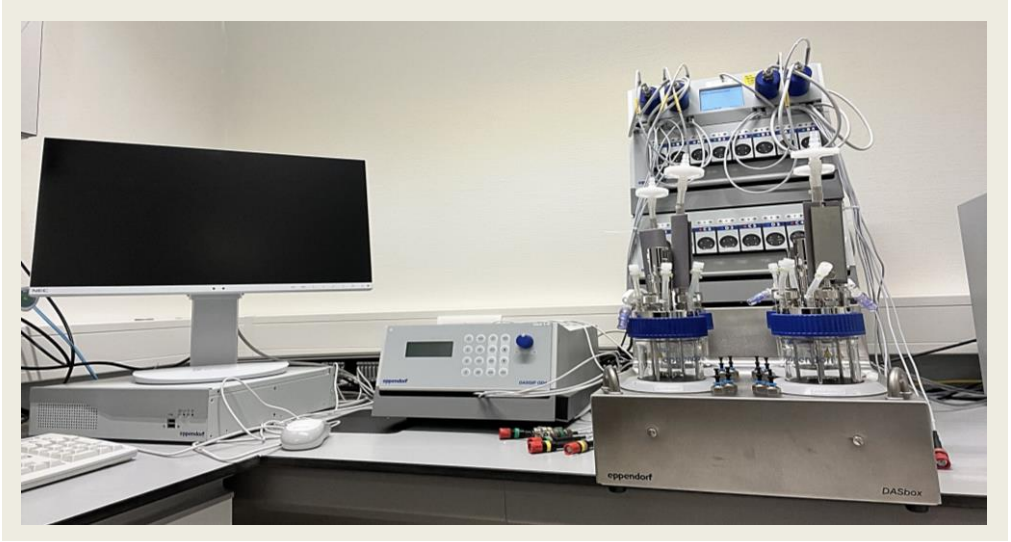

Figure 8. DASbox system. Four-fold system supporting working volumes of 60 to 250 ml and real time monitoring of the critical experimental conditions.

7. PhD project self-evaluation

The two main objectives of this PhD were i. to study the dynamic processes of the developing caecal microbiota of commercial broiler chickens in relation to ESBL-*E. coli* colonisation and ii. to develop an *in vitro* caecal model that allows studying the effect of antibiotics and microbiota-targeted interventions on the horizontal AMR gene transfer dynamics in the *in vitro* chicken caecal microbiota

To facilitate the achievement of these objectives, the PhD was set up in two components: *in vivo* and *in vitro* studies. The *in vivo* studies were performed with samples collected from broiler chickens from commercial Dutch farms. This characteristic of the *in vivo* study was a plus since it allowed us to gain insights into the natural dynamic of ESBL colonisation occurring on a farm and its relationship with the changes in the microbial community composition of broiler chickens over time. This advantage of the study also implied taking risks, like the inability to sample due to in-farm infection disease outbreaks.

The COVID-19 pandemic from 2020-2021 and an extensive Avian Influence outbreak in 2022 impeded the farm visits and sample collection, considerably delaying the completion of the second study on the relationship between chicken gut microbiota development of slow-growing broilers and ESBL-*E. coli* colonisation. As a result, a deviation from the work plan was considered. Namely, samples were collected from research facilities instead of commercial broiler farms under the same conditions. The animals were not subjected to additional handling or stress, and as such, we believe the project's ethics did not deviate from the plan.

Similarly to the *in vivo* work, the *in vitro* work was also considerably affected by the COVID-19 lockdowns during the stages of equipment set up and training. The set-up of an *in vitro* chicken caecal model was a high-risk and challenging process which required considerable programming work and optimisation. Although the *in vitro* model produced preliminary expected data, recurrent technical difficulties (software/hardware-related issues) hindered the completion of the experiments to study the horizontal gene transfer dynamics in the *in vitro* chicken caecal microbiota after colonisation with ESBL *E. coli* strains and microbiota-targeted interventions in the proposed time. This problem was tackled by acquiring new equipment with a dedicated anaerobic module expected to reach anaerobic conditions more reliably. The new equipment was installed in February 2023, and transfer of knowledge, test experiments and optimisation are currently being carried out. Delayed *in vitro* intervention experiments are expected to take place in the third quarter of 2023.

In anticipation of the start of the *in vitro* intervention experiments, the PhD student performed a short-term mission at the One Health Antimicrobial Resistance (OHAR) research group lead Prof. Luca Guardabassi at the University of Copenhagen. During this period, the PhD student learned molecular cloning techniques to label the chromosome and ESBL plasmids of *E. coli* strains with fluorescent reporter proteins. Such strains will be used to track the spread of ESBL plasmids in the *in vitro* chicken caecal microbiota.

Despite of the adverse situations due to COVID-19 pandemic, Avian Influenza outbreaks, and technical issues, the PhD project has produced valuable data and has contributed to understanding the chicken caecal microbiota development dynamics in relation to ESBL-*E. coli* colonisation during the broiler

chicken lifespan. As well, as to set up a reproducible *in vitro* chicken model that will allow further experimental work and the answer to microbiota and AMR-related research questions.

8. Progress of the project: milestones and deliverables

Deliverables

PhD reference	PhD Project deliverable number	Deliverable name *(Original name, if different from the actual one)*	Delivery date from AWPs (month)	Date delivered (month)	Comments *(Please mention: public or confidential, Zenodo reference, reason and justification of delay, other comments)*	Integrative categories*
	D1	Manuscript on preliminary findings for the relationship between chicken gut microbiota development and ESBL-*E. coli* colonisation.	36	45	Public: zenodo 7057449	1
	D2	Manuscript on the relationship between chicken gut microbiota development of slow-growing broilers and ESBL-*E. coli* colonisation.	56		Public Due to the COVID-19 pandemic in 2020 and an extensive non-seasonal Avian Influenza outbreak in the Netherlands, no farm sampling has been possible.	1
	D3	Manuscript describing the results of testing *in vitro* ESBL *E. coli* intervention strategies.	60		Public	6
	D4	Manuscript to describe the host range of ESBL plasmids from *E. coli* into the *in vitro* microbiota and strategies to lower plasmid spread.	68		Public	6

** Categories of Integrative activities : 1. Design and implementation of surveillance and control activities; 2. Harmonised protocols and applied best practice; 3. Databases of reference materials and data, incl. metadata; 4. Standardised data formats, aligned data analysis for interpretation of surveillance data; 5. Sharing and communication of surveillance data; 6. Sharing of best intervention activities); 7. Prevention: aligned use of facilities and models; 8. Other (please specify);*

Milestones

PhD reference	Milestone number	Milestone name	Delivery date from AWPs	Actual delivery date	Achieved	Comments
	M1	Molecular technique training and 16S barcode sequencing.	22	22	Yes	
	M2	Attend a course on analysis for 16S barcode sequencing.	24	24	Yes	
	M3	Visit APHA for training on *in vitro* chicken gut model.	24		Yes	The training was performed online.
	M4	Perform 16S barcode sequencing on currently collected samples.	27	27	Yes	
	M5	Perform 16S rRNA gene analysis on initial experiment	30	31	Yes	
	M6	Perform initial test runs on *in vitro* gut model to determine CFU for reliable ESBL colonisation.	36	-	No	Due to technical difficulties in the set-up of the model, this will be carried out after the installation/optimisation of new equipment, expected in May-June 2023.
	M7	Write a manuscript on the relationship between chicken	36	56	Yes	Published on M56

		gut microbiota development and ESBL-*E. coli* colonisation.				
	M8	Perform 16S rRNA gene sequencing and analysis of caecal samples from OHEJP VIMOGUT.	50	-	No	Farm visits were rescheduled due to COVID-19 and avian influenza.
	M9	Manuscript on the relationship between chicken gut microbiota development of slow-growing broilers and ESBL-*E. coli* colonisation	56	-	No	Farm visits for sampling were rescheduled due to COVID-19 and avian influenza.
	M10	Experiments in the *in vitro* model for ESBL *E. coli* colonisation intervention strategies.	60	-	No	Due to technical difficulties in the set-up of the model, this will be carried out after the installation/optimisation of new equipment, expected in May-June 2023.
	M11	Experiment in the *in vitro* model on the horizontal transfer of ESBL plasmids within the caecal microbiota	60	-	No	Due to technical difficulties in the set-up of the model, this will be carried out after the installation/optimisation of new equipment, expected in May-June 2023.
	M12	Experiments in the *in vitro* model to evaluate the effect of microbial interventions on the spread of ESBL plasmids	64	-	No	Due to technical difficulties in the set-up of the model, this will be carried out after the installation/optimisation of new equipment, expected in May-June 2023.
	M13	Write a manuscript describing the results of the experiments on the effects of antibiotics and intervention strategies on	68	-	No	Expected after the installation of the new system and collection of data from the *in vitro* experiments.

		the transmission of ESBL plasmids in the *in vitro* caecal microbial community.				

9. Interactions with JRPs/JIPs or with external (global, EU, national or regional) relevant projects or initiatives such as national action plans (AMR, Zoonoses etc.), national and international surveillance programmes.

Data collected during OH-EJP JRP ARDIG is being further analysed during the VIMOGUT project.

10. Interactions with OHEJP stakeholders

1. During OH-EJP ARDIG, samples from broiler farms were collected and further analysed during the PhD project VIMOGUT. https://onehealthejp.eu/projects/antimicrobial-resistance/jrp-ardig

2. The *in vitro* chicken caecal model that was developed for VIMOGUT is currently also employed for the JPI-AMR STRESST project. Expertise that was gained through VIMOGUT is further utilised for the development of an *in vitro* waste water model to study the transfer of AMR genes. https://www.jpiamr.eu/projects/stresst/

3. Co-funding for the VIMOGUT project was received from the Dutch Ministry of Agriculture, Nature and Food Quality. Results of the VIMOGUT project have been shared with the ministry throughout the project's lifetime.

4. Link with national projects:
    a. National project for AMR monitoring in livestock in the Netherlands.
    b. National project on ESBL-prevalence in veal calves in the Netherlands.
    c. National project on epidemiology of ESBL-carrying plasmids using long-read sequencing in the Netherlands.
    d. National AMR monitoring in livestock project code: WOT-01-002-038: https://www.wur.nl/nl/onderzoek-resultaten/onderzoeksprojecten-lnv/soorten-onderzoek/kennisonline/antibioticum-resistentie-5.htm

11. Added value and benefits during PhD resulting from being part of the OHEJP doctoral programme and consortium

Being a OH-EJP PhD student opened up different channels and opportunities, from gaining and training skills and competences, to enriching the student's scientific knowledge and network.

Early in 2020, VIMOGUT collaborated with researchers from the Helmholtz Centre for Environmental Research – UFZ in Leipzig, Germany. This collaboration strengthened our knowledge on microbiome data analysis and generated fruitful ideas about studying the chicken caecal microbiota development on relation to colonisation with resistant. This work is currently published as a research article in the scientific journal *Animal microbiome*.

Moreover, valuable knowledge was exchanged with the Animal Plant Health Agency (APHA) in UK to establish an *in vitro* chicken gut model capable of reproducing the main physiological conditions of the caeca and maintain the main microbial members that populate this organ.

Other fruitful collaborations were set up with the One Health Antimicrobial Resistant (OHAR) group of the University of Copenhagen via a short-term mission (STM) in 2022. During the STM, the PhD student learned bacterial cloning techniques and developed and strengthened her lab skills to produce dual fluorescently labelled bacterial strains. The dual labelling of bacteria is essential to carry out the *in vitro* experiments that aim to study the effect of antibiotics and microbiota interventions in the horizontal spread of Extended Spectrum ß-Lactam (ESBL) genes. The output of this collaborative work is expected to be published as a research article in a scientific journal.

12. Transferrable skills and Training

Name of Training Event	Topic	Dates (DD/MM/YY)	Organising Institute
Scientific integrity	Skills and competences	03/03/23	Wageningen Graduate School
Supervising BSc & Master students	Skills and competences	09-10/02/23	Wageningen Graduate School
Workshop on human microbiome & e-DNA research for public health & the environment	Microbiome in health and disease and as tool to monitor diversity	26-27/09/22	National Institute for Public Health and the Environment (RIVM)
Microbial Ecology course	Microbial Ecology	19-24/06/22	PE&RC graduate school
Short Term Mission at the University of Copenhagen	Bacterial cloning - AMR	30/04/22 - 09/07/22	OH-EJP
Poster and pitching	Skills, competences and career development	10/03/22, 31/03/22, 07/04/22	Wageningen Graduate School
Workshop on plasmids as vehicles of antimicrobial resistance spread	AMR	21-25/03/22	International Centre for Theoretical Physics
Reviewing a scientific manuscript	Skills, competences and career development	17/02/22	Wageningen Graduate School
R Markdown	Data analysis	05-06/07/21	VLAG – WSG
Multivariate analysis course	Statistical analysis	23-29/06/21	PE&RC – Wageningen Graduate School
Mindful productivity for PhD Candidates	Personal development	28/05/21	PE&RC - Wageningen Graduate School

Effective and efficient verbal communication in academia and beyond	Communication	26/05/21	PE&RC - Wageningen Graduate School
Applications of omics technologies in poultry health and productivity: where are we now?	Omics	22/04/21	*IHSIG*
Global Tricycle Surveillance ESBL *E. coli*	AMR	03/03/21	WHO
7th OHEJP cogwheel workshop	Science	25/02/21	OH-EJP
Imposter syndrome	Personal development	24/02/21	CACTUS
Scicom IG meeting	Science Communication	16/02/21	WUR
Metagenomics webinar (DADA2)	Data analysis	21/01/21	Loop Genomics
Peer review discussion meeting	Communication	12/01/21	GSS-WUR
Cogwheel workshop	Science	25/11/20	OH-EJP/Horinzon 2020
Writing course	Scientific writing	09-10/09/20	Leipzig University
Basic cultivation course	Bioengineering	12-15/10/20	Applikon
Advanced community ecological data analysis using vegan	Statistics/bioinformatics	09/07/20	University of Regina, Canada (online)
Introduction to multivariate data analysis using vegan	Statistics/bioinformatics	07/07/20	University of Regina, Canada (online)

13. Ethical Reviews

Comments of Ethics Advisors, January 2020	Comments PhD Project Supervisor, mid-2020	Comments of Ethics Advisors, October 2020
This project is using broiler chickens at conventional farms. Further details are needed on the researcher's interaction and 'use' of a legal animals (e.g. the broiler chickens). If these are not experimental animals as defined in Directive 2010/63/EU they are still legal animals through national animal welfare laws so please comment on any implications for the the broiler chickens from sampling etc. Please describe how the animals'	During the VIMOGUT project, sampling at conventional broiler farms and possibly slaughter houses will be carried out. If samples are taken at slaughter houses, the intestinal tract of animals will be collected after slaughter and evisceration are carried out. No changes are made to the standard procedures of the site and no additional animals are slaughtered for the benefit of the research. For the sampling at farms, only	

welfare are protected and considered (e.g. if the chickens are affected when taking samples, even if the work is dealing with faeces as these types of study can, this can still include restricting the animals or manipulating diets, etc, all which can have an impact on the animals). Please provide a statement on the 3Rs aspects of this work If Ethical Approval is required please state which Research Ethics Committee this will be sent to.	conventional broilers will be used with no restrictions or manipulation of the animals' diets. Animals will not be sacrificed for the research carried out for VIMOGUT unless approval has been given by the local animal welfare committee (IvD) at WUR and the national board on animal experiments (CCD), as per local guidelines. Sampling at conventional broiler farms will include the collection of fresh droppings for which animals may be briefly isolated to relieve themselves. Handling of the animals will only be performed after careful instruction and under the constant supervision of a trained veterinarian. As per local law in the Netherlands, the 3Rs are always considered when animal experiments or on-site sampling is performed. The *in vitro* model that is set up during VIMOGUT is part of the strategy to replace the need for animal experiments in microbiota research. To refine the experiments and reduce the number of animals that are sacrificed, fresh droppings will be used for this study instead of caecal content of the animals, unless there is there is a clear need for the use of caecal content. As mentioned above, permission will be sought from the local animal welfare committee IvD and the national board on animal experiments CCD.	

14. Scientific Publications

Publication date	Publication title	Authors	DOI reference	Zenodo reference	Is OHEJP acknowledged?	Is it a Green Open Access? *please specify embargo length	Is it a Gold Open Access?
19/08/22	Succession in the caecal microbiota of developing broilers colonised by extended-spectrum β-lactamase-producing *Escherichia coli*	Ingrid Cárdenas-Rey, Teresita d. J. Bello Gonzalez , Jeanet van der Goot , Daniela Ceccarelli, Gerwin Bouwhuis, Danielle Schillemans, Stephanie D. Jurburg, Kees T. Veldman, J. Arjan G. M. de Visser and Michael S. M. Brouwer	https://doi.org/10.1186/s42523-022-00199-4	https://zenodo.org/record/7057449#.YxiUTHZByUk	Yes		Yes

15. Additional Outputs

Short Term Mission completion: "Construction of double-labelled *E. coli* strains to study the effect of antibiotics and interventions on horizontal ESBL genes transfer in the chicken's caecal microbiome" at the One Health Antimicrobial Resistant group at the University of Copenhagen.

https://onehealthejp.eu/short-term-missions-2022/
https://zenodo.org/record/7461849#.Y6F1B3bMKUk

16. Specific outcomes to highlight in dissemination and communications

The research article titled: **Succession in the caecal microbiota of developing broilers colonised by extended-spectrum β-lactamase-producing *Escherichia coli*** was published in the scientific journal Animal Microbiome on August 19 2022. In this research article, we assessed the role of ESBL-*E. coli* in the successional dynamics of the caecal microbiota in developing commercial broilers. Our findings suggest that ESBL-*E. coli* is associated with mild but consistent alpha diversity reductions and transient bacterial compositional differences. We also documented the prevalence trend and clonal spread of ESBL-*E. coli* in a broiler farm and pointed to the farm environment as a potential source for ESBLs.

Results from the ongoing *in vivo* and *in vitro* studies mentioned above are expected to be published as peer-reviewed research articles in scientific journals. Other dissemination and communications activities are listed in section 15 and 18.

17. One Health impact

To effectively combat the health threat of AMR for humans and livestock animals, we need to understand better how AMR genes arise and spread within and between host species and the general environment. VIMOGUT contributed to this aim in several complementary ways.

First, we have studied the prevalence dynamics of resistant bacteria like ESBL-*E. coli* in natural conditions. We have shown how the clonal spread of AMR genes occurred in a broiler population. Moreover, and confirming those findings, our ongoing longitudinal study on ESBL/AmpC plasmid epidemiology suggests that despite routine cleaning, the same ESBL-*E. coli* clones are maintained in the environment and detected in different production rounds. These results emphasize the need for further development and improvement of farm biosecurity and management via recommendations or policies by the national authorities.

Second, we have considered an important workhorse in the battle against AMR: the gut microbiota. Alternative interventions besides antibiotic usage reduction need to be explored, like understanding and modulating the gut microbiota. Modifying such complex microbial communities can help tackle the spread of AMR bacteria and improve the health of animals and humans. We have shown that changes in microbiota are observed during the natural colonization of resistant bacteria and therefore offer an opportunity for modulation and natural exclusion of pathogens and resistant bacteria. This could be considered a One Health approach where the animal, the human, and the environment’s health would benefit.

Third, we have developed an *in vitro* caecal model that will allow us to determine the fate of plasmid-encoded AMR genes and further study microbiota-targeted interventions that might help to reduce the spread of these genes in complex microbial communities like the chicken gut. Additionally, the *in vitro* chicken caecal model is a step ahead in doing sustainable research. Despite its limitations, this cost-efficient tool allows ample research with controlled parameters and contributes to using fewer resources, including the unnecessary use of animals in the first stages of research or hypothesis testing.

Ultimately, VIMOGUT outcomes will support the development of new intervention strategies to hinder the spread of AMR in animals and subsequently in humans and environment. This will result in the production of safer food with a lower risk of transfer of AMR genes from broiler chickens to humans via the food chain or alternative routes. This One Health approach is supported by all stakeholders mentioned, including EFSA, ECDC, WHO, FAO, and the Ministry for Agriculture, Nature and Food Quality in the Netherlands.

18. List of dissemination and communication activities

Name of the activity:	*FEMS 2023 Congress of European Microbiologists (poster presentation)*		
Date:	*9-13th July 2023*		
Place:	*Hamburg, Germany*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		**Participation to a Conference**	*Yes*
Organisation of a Workshop		**Participation to a Workshop**	
Press release		**Participation to an Event other than a Conference or a Workshop**	
Non-scientific and non-peer-reviewed publication (popularised publication)		**Video/Film**	
Exhibition		**Brokerage Event**	
Flyer		**Pitch Event**	*Yes*
Training		**Trade Fair**	
Social Media		**Participation in activities organised jointly with other H2020 projects**	
Website		**Other**	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	**>500**	**Media**	
Industry		**Investors**	
Civil Society		**Customers**	
General Public		**Other**	
Policy Makers			

Name of the activity:	*OH-EJP 3-minute thesis competition at OH-EJP ASM 2022*		
Date:	*11-04-22*		
Place:	*Orvieto, Italy*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		**Participation to a Conference**	*Yes*
Organisation of a Workshop		**Participation to a Workshop**	
Press release		**Participation to an Event other than a Conference or a Workshop**	
Non-scientific and non-peer-reviewed publication (popularised publication)		**Video/Film**	
Exhibition		**Brokerage Event**	

Flyer		***Pitch Event***	*Yes*
Training		***Trade Fair***	
Social Media		***Participation in activities organised jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***>500***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Poster presentation at OHEJP ASM 2022*
Date:	*11-13/04/22*
Place:	*Orvieto, Italy*

Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organised jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*

Scientific Community (Higher Education, Research)	***>500***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*PhD round table OHEJP ASM 2022*		
Date:	*13/04/22*		
Place:	*Orvieto, Italy*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organised jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***>500***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	

General Public		*Other*	
Policy Makers			

Name of the activity:	Poster carousel, Microbial ecology course.		
Date:	19-24 June 2022		
Place:	Nunspet, The Netherlands		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		*Participation to a Conference*	
Organisation of a Workshop		*Participation to a Workshop*	Yes
Press release		*Participation to an Event other than a Conference or a Workshop*	
Non-scientific and non-peer-reviewed publication (popularised publication)		*Video/Film*	
Exhibition		*Brokerage Event*	
Flyer		*Pitch Event*	Yes
Training		*Trade Fair*	
Social Media		*Participation in activities organised jointly with other H2020 projects*	
Website		*Other*	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categories			
	Number		*Number*
Scientific Community (Higher Education, Research)	**50**	*Media*	
Industry		*Investors*	
Civil Society		*Customers*	
General Public		*Other*	
Policy Makers			

Name of the activity:	*OH-EJP 3-minute thesis competition at OH-EJP ASM 2021*		
Date:	*10-06-21*		
Place:	*Online*		
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories			
	Yes / No		*Yes / No*
Organisation of a Conference		*Participation to a Conference*	*Yes*
Organisation of a Workshop		*Participation to a Workshop*	
Press release		*Participation to an Event other than a Conference or a Workshop*	
Non-scientific and non-peer-reviewed publication (popularised publication)		*Video/Film*	
Exhibition		*Brokerage Event*	
Flyer		*Pitch Event*	*Yes*

Training		***Trade Fair***	
Social Media		***Participation in activities organised jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*
Scientific Community (Higher Education, Research)	***1000***	***Media***	
Industry		***Investors***	
Civil Society		***Customers***	
General Public		***Other***	
Policy Makers			

Name of the activity:	*Poster presentation at OHEJP ASM 2021*
Date:	*09-06-21 / 11-06-21*
Place:	*Online*
Specify the Dissemination and Communication activities linked to the One Health EJP project for each of the following categories	

	Yes / No		*Yes / No*
Organisation of a Conference		***Participation to a Conference***	*Yes*
Organisation of a Workshop		***Participation to a Workshop***	
Press release		***Participation to an Event other than a Conference or a Workshop***	
Non-scientific and non-peer-reviewed publication (popularised publication)		***Video/Film***	
Exhibition		***Brokerage Event***	
Flyer		***Pitch Event***	
Training		***Trade Fair***	
Social Media		***Participation in activities organised jointly with other H2020 projects***	
Website		***Other***	
Communication Campaign (e.g. Radio, TV)			
Specify the estimated number of persons reached, in the context of this dissemination and communication activity), in each of the following categoriesS			
	Number		*Number*

Scientific Community (Higher Education, Research)	*1000*	*Media*	
Industry		*Investors*	
Civil Society		*Customers*	
General Public		*Other*	
Policy Makers			