SCIENTIFIC SYMPOSIUM

"ANTIMICROBIAL RESISTANCE: A CHALLENGE FOR PUBLIC HEALTH, ANIMAL HEALTH AND THE ENVIRONMENT"

June 25th, 2026, 9 am – 12 am

Brussels (Pacheco center) – webinar

Program

Registration and coffee from 9 am

Posters with selected scientific communications will be displayed on site.

9h30	Welcome by Prof. Dominiek Maes (Ghent University and chair of AMCRA)
9h35	**Keynote presentation: The role of animal clinics in the selection and spread of antimicrobial resistance, by Prof. Dr. Simone Schuller (Vetsuisse Faculty, University of Bern, Switzerland)**
10h15	Adapt and Conquer: can pathogens evolve resistance to probiotics? (Hans Steenackers, KU Leuven)
10h30	Enabling wound monitoring through Real-time Fluorescence Lifetime Imaging (Simone Janssen, UGent)
10h45	Coffee break and poster session

11h15	Second session chaired by Prof. Damien Thiry (University of Liège)
11h15	Retrospective study of bacteriological results of equine uterine samples in Flanders between 2015 and 2025 (Manon Janssen, UGent)
11h30	Biosecurity implementation Gaps and antimicrobial resistance circulation in companion animal veterinary clinics (Junjia He, UGent)
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12h	**Poster prizes announcement** and closing remarks by Prof Damien Thiry
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Abstracts for oral presentations

Keynote presentation: The role of animal clinics in the selection and spread of antimicrobial resistance

Prof. Dr. Simone Schuller

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Simone Schuller is a veterinarian, she graduated at the University Munich, Germany. Between 2002 and 2005 she completed a Residency in small animal internal medicine at the Faculty of Veterinary Medicine in Liège. Between 2005 and 2013 she was Lecturer small animal medicine at the University College Dublin and completed a PhD in infection biology.
Since 2014, she is Professor at the Vetsuisse Faculty of the University of Bern, where she works in clinic, teaching and research.
Her interests lie in the field of respiratory and infectious diseases. In recent years, she has worked extensively on the topic of antibiotic resistance and hospital hygiene as part of research projects and has been involved in the creation of the Swiss guideline for the prudent use of antibiotics in small animal medicine.

Adapt and Conquer: Can Pathogens Evolve Resistance to Probiotics?

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Introduction

In recent years, probiotics have emerged as a promising microbial technology to combat bacterial infections and reduce antibiotic use in animal husbandry. Their anti-pathogenic activity is often multifactorial, including competition for shared resources (exploitation competition) and secretion of antimicrobial compounds (interference competition). As such, probiotics are increasingly explored as evolutionarily informed alternatives to conventional antimicrobials in the context of antimicrobial resistance (AMR). However, much like antibiotic resistance, pathogens may adapt to probiotics, which could reduce their effectiveness. Additionally, as living organisms subject to natural selection, probiotics themselves may adapt in response to competition with a pathogen, creating co-evolutionary dynamics.

Materials and methods

To better understand these co-evolutionary dynamics between pathogens and probiotics, we co-evolved the commercial probiotic *Escherichia coli* Nissle 1917 (EcN) and the enteric pathogen *Salmonella* Typhimurium for approximately a month in different environments. As both probiotics and *Salmonella* are highly relevant in livestock production systems, understanding their long-term evolutionary interactions is essential for the sustainable implementation of probiotic-based interventions in animal health.

Results and conclusion

In nutrient-rich liquid cultures, where EcN primarily inhibits *Salmonella* via resource competition, *Salmonella* can rapidly adapt by acquiring metabolic mutations that enhance nutrient scavenging. In biofilms, the anti-Salmonella activity of EcN is less dependent on resource competition but primarily mediated by small antimicrobial peptides or microcins. Evolution in these structured biofilms seems to be more complex, with local sectors emerging that sometimes are overtaken again by the other competitor over time. We are currently characterizing isolates recovered from these evolved biofilms to investigate whether *Salmonella* has acquired resistance to EcN's microcins, and whether EcN has in turn acquired improved anti-*Salmonella* activity.

These findings underscore the potential for pathogens to adapt to probiotics and highlight the importance of studying these co-evolutionary interactions when developing alternative antimicrobial strategies. Understanding both pathogen adaptations and probiotic counter-adaptations provides critical insights into resistance risks and offers opportunities for designing next-generation probiotics with enhanced anti-pathogenic properties for applications in both human and veterinary medicine.

Enabling Wound Monitoring Through Real-time Fluorescence Lifetime Imaging.

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Introduction

In veterinary practice, wounds in companion animals are a common indicator for antimicrobial use. However, treatment decisions often rely on subjective assessment of the wound bed, which may lead to inappropriate antibiotic use, contributing to antimicrobial resistance. Although recent legislation has improved antimicrobial stewardship, variability in prescribing habits persists. Current diagnostic approaches, such as *in vitro* bacterial identification, remain important but require samples to be sent out for laboratory processing and do not provide real-time feedback during consultation. Existing imaging techniques can detect bacterial burden but lack specificity. Fluorescence lifetime (FL)-based imaging of endogenous autofluorescence offers a novel approach to assess wound status by capturing metabolic and biochemical changes in tissue. This technique has already shown potential for bacterial detection and subtype differentiation in microscopy (FLIM), suggesting it may support more rational antimicrobial use. In this abstract, we present macroscopic observations from a single canine trauma case, illustrating changes in wound bed FL before and after debridement. An FWO senior research project has been funded to investigate the relationship between FL parameters and bacterial load in dogs with acute and chronic wounds.

Materials and methods

In vivo autofluorescence measurements will be acquired in dogs with acute and chronic wounds. Images are made from the wound bed at different stages of treatment, before and after cleansing and debridement. Both fluorescence intensity (FI) and FL signals are recorded using multispectral macroscopic imaging to evaluate tissue condition and potential bacterial presence. Excitation is performed using a 405 nm pulsed laser, while emission is detected across multiple wavelength bands. Imaging is performed using the VUB tauCAM system (Fig. 1a), developed for macroscale FL imaging. Findings will be correlated *ex vivo* with bacterial cultures, FLIM, histopathology, and thermography. This multimodal approach aims to evaluate FL parameters as indicators of bacterial burden and tissue conditions.

Results and conclusion

Preliminary results indicate that FL imaging provides additional information on the wound bed status. While FI showed limited specificity across the wound bed, FL measurements revealed distinct spatial variations associated with tissue condition (Fig. 1b). The FL values changed after wound debridement, suggesting sensitivity to alterations in the wound bed environment. Further work will focus on the determination of the relationship between FL values and bacterial presence and identification.

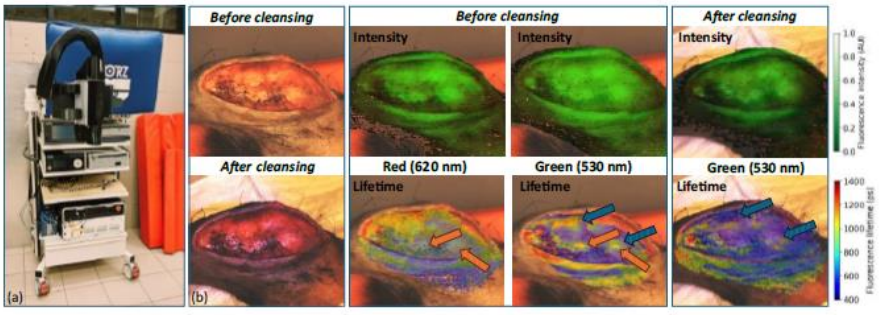

Fig. 1 (a) tauCAM system **(b)** FI and FL images of a deep wound on the right hind leg of a female dog, 3 days after being hit by a car, before and after cleansing (i.e., saline) and sharp debridement. Arrows: FL differences before and after cleansing (blue), and between spectral bands (orange).

The findings support the potential of this non-invasive tool to provide objective, real-time information on wound status. By improving insight into bacterial involvement, this approach may enable more targeted antimicrobial use and contribute to antimicrobial stewardship in veterinary practice, with potential reduction of unnecessary antibiotic use and mitigating antimicrobial resistance in veterinary and human medicine.

Suggested literature: Qazi F et al. Microbes Infect. 2024;26(3):105263

Retrospective study of bacteriological results of equine uterine samples in Flanders between 2015 and 2025

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Introduction

Endometritis is a major cause of subfertility in mares and is characterized by persistent inflammation of the endometrium, often resulting from bacterial contamination. Although a physiological inflammatory response after breeding is normal, impaired uterine clearance can lead to persistent infection.
The most frequently isolated pathogens include *Streptococcus spp*., *Escherichia coli*, and *Staphylococcus spp*. In addition to the presence of pathogens, antimicrobial resistance represents a growing challenge in veterinary medicine. International studies have demonstrated considerable variation in both pathogen prevalence and resistance patterns. However, recent data for Flanders remains limited. The aim of this study was to analyze the prevalence of bacterial pathogens and their antimicrobial resistance patterns in uterine samples from mares in Flanders, as well as to evaluate their evolution over the period 2015–2025.

Materials and methods

A retrospective study was conducted using bacteriological data from endometrial samples (N=4001) obtained from mares with reproductive disorders in Flanders between January 2015 and December 2025.
Uterine samples were collected using swabs or lavages by veterinarians and subsequently sent to a specialized lab for bacteriological analysis. Bacterial identification was performed using MALDI-TOF and biochemical testing. For each isolate, antimicrobial susceptibility was determined using Vitek 2 systems or disk diffusion methods.
Data were analyzed based on bacterial species, Gram classification, and resistance patterns. Statistical analyses included chi-square tests to assess trends in prevalence and species distribution, as well as correlation analyses to evaluate changes in antimicrobial resistance over time.

Results and conclusion

The majority of isolates were Gram-positive bacteria (n=2295). *Streptococcus spp.* was the most frequently isolated pathogen (n = 1361), followed by *Escherichia coli* (n = 699) and *Staphylococcus spp.* (n = 416). A shift in pathogen prevalence was observed over time ($P < 0.001$), with a relative increase in Gram-negative bacteria (35% in ‘17 vs. 57% in ‘25).

Regarding antimicrobial resistance, an overall increase was observed across multiple bacterial species. *Streptococcus spp*. remained largely susceptible to β-lactam antibiotics (88,5%) but showed increasing resistance to alternative agents. In *Escherichia coli*, a clear rise in resistance to commonly used antibiotics was detected (ampicillin, gentamycin, enrofloxacin,…). Other Enterobacteria, such as *Enterobacter spp*. and *Klebsiella spp.* also exhibited increasing resistance. Among non-Enterobacteria, f.i. *Pseudomonas spp.*, demonstrated a clear resistant profile against multiple antibiotics.
These results indicate a shift in the bacterial population associated with equine endometritis and an increasing trend in antimicrobial resistance in Flanders. This highlights the importance of targeted therapy based on bacteriological examination and antimicrobial susceptibility testing, as well as the need for responsible antibiotic use and continuous monitoring.

Biosecurity Implementation Gaps and Antimicrobial Resistance Circulation in Companion Animal Veterinary Clinics

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Introduction

Companion animal veterinary clinics are viewed as potential antimicrobial-resistant bacteria reservoirs. Yet environmental contamination patterns and biosecurity practices remain poorly characterized. This study investigated these patterns and evaluated biosecurity measures to identify critical infection prevention control points.

Materials and methods

Environmental sampling (n=423) was conducted across 12 veterinary facilities at three timepoints: before daily cleaning and disinfection, after daily cleaning and disinfection, and after 3-5 client visits. Samples were collected from high-touch surfaces using sterile sponge-sticks and processed for isolation of indicator bacteria (E. coli, E. faecalis, and E. faecium). Antimicrobial susceptibility testing was performed using micro-dilution methods. Biosecurity compliance was assessed through structured questionnaires.

Results and conclusion

Of the 423 environmental samples, 40.7% were positive for at least one indicator bacterium. Enterococci showed higher isolation rates than E. coli, with E. faecium being most prevalent. Multi-drug resistance was detected in substantial proportions of isolates, with E. faecium showing higher resistance rates than E. faecalis. Critically, bacterial isolation rates increased after cleaning procedures in several practice areas, suggesting poor cleaning procedures and potential redistribution rather than elimination of contamination. Biosecurity assessment revealed significant implementation gaps, with poor adherence to "onward march" protocols and inadequate quality evaluation of cleaning procedures.

This study confirms the presence of (multi) resistant bacteria in the studied companion animal clinics. While most clinics have C&D plans and basic infection control procedures, the inconsistent application of these protocols compromises infection control and the observed redistribution of resistant bacteria post-cleaning highlights an urgent need for standardized, evidence-based biosecurity interventions.

Antibiotic Resistance in Aquatic Environmental Hotspots

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Introduction

Antibiotic resistance poses a severe threat to the treatment of infectious diseases and continues to expand globally, largely driven by the overuse and misuse of antibiotics. In the context of the One Health approach, it is crucial to recognize the interconnection of human and veterinary medicine and the role of the environment in the spread of antimicrobial resistance. Contamination of the aquatic environment with antibiotic residues and resistant bacteria can occur through several pathways and is, among others, associated with the use of manure as fertilizer on arable land, ultimately leading to runoff into surrounding water bodies. Even at low concentrations, antibiotic residues can promote the development and persistence of resistance by exerting selective pressure on bacterial populations. When antibiotic-resistant bacteria from the environment are taken up by humans, resistance genes may be transferred to bacteria belonging to the human gut microbiota, including potential pathogens.

Materials and methods

This research focused on two hotspots in Belgium; manure-derived pollution through run-off to surface water and groundwater and harbor environments with input from various sources. The sampled freshwater locations included 50 surface water locations (part of the Manure Action Plan monitoring network), sampled before and after fertilization, and 50 groundwater wells. Furthermore, 23 locations across the harbors of Oostende and Nieuwpoort, which included both water and sediment sampling, were selected. In this research, methods based on liquid-chromatography coupled to a mass spectrometer were developed to detect and quantify antibiotic residues across water and sediment samples. Indicator organisms, Escherichia coli and Extended spectrum β-lactamase (ESBL)-producing E. coli, were isolated and tested for their antibiotic susceptibility to evaluate the resistant phenotype originating from fecal contamination. A selection of 48 isolates was subsequently analyzed by short-read whole genome sequencing to screen for genotypic resistance, strain diversity and virulent properties of ESBL-producing E. coli in aquatic environments. Comparing the occurrence of antibiotic residues and observed resistance within the same sample enables identification of antibiotic-specific risks and differences between sample types.

Results and conclusion

The presence of a wide range of antibiotic residues, though at low concentrations in agricultural freshwater, highlights the extensive spread of these substances. Notably, the frequent occurrence of sulfonamides and lincomycin in surface waters raises concerns as their concentrations occasionally exceed the predicted no-effect levels for antibiotic resistance selection. Additionally, resistance patterns in E. coli indicate increased resistance to sulfamethoxazole following fertilization ($p < 0.05$). Although antibiotic contamination in groundwater is less prevalent, antibiotic resistance remains widespread. In harbors, sediments contained more and a higher concentration of antibiotic residues than water samples. There was a link between antibiotic accumulation in sediments and selective pressure favoring resistant bacterial populations, particularly for quinolones.

In addition, ESBL-producing E. coli consistently exhibited heightened phenotypic resistance levels, not limited to β-lactam antibiotics, compared to the isolated non-ESBL-producing E. coli. The detection of

resistance to critical last-resort antibiotics such as carbapenems and colistin further emphasizes the urgency of addressing antibiotic resistance in environmental contexts.

Whole-genome sequencing expanded on these insights on a genetic level and unraveled the diversity and dissemination of ESBL-producing E. coli in aquatic environments. The typing results showed substantial variability among isolates without differences between sample types, reflecting their widespread dissemination in the environment. ESBL-producing E. coli served as important reservoirs of resistance to multiple antibiotic classes, including critically important agents, with resistance determinants frequently carried on mobile plasmids and associated with integrons, insertion sequences and transposons

This study highlights the need for continued monitoring and the implementation of legislation addressing antibiotic pollution and resistance in aquatic ecosystems.

Abstracts for poster presentations

What's in the code of Salmonella Infantis? Resistance in the Belgian Poultry Sector

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Introduction

An important human pathogen, which is persistently present in the Belgian broiler sector, is *Salmonella* Infantis (SI). It is the fourth most common *Salmonella* serovar causing salmonellosis in Europe and is known to possess a megaplasmid (pESI). According to literature, this plasmid lends SI antibiotic resistance and biocide tolerance as well as other persistent characteristics. The pESI plasmid was first discovered in Israel in 2014 by Aviv *et al.* (2014), but soon spread through Europe and the world. This research analyzed the antibiotic resistance genotype of SI isolates from the Belgian poultry sector and looked into the contribution by the megaplasmid.

Materials and methods

A selection of 50 SI strains originating from the broiler sector in Belgium were sequenced using Oxford Nanopore Technology. Raw data was filtered targeting a coverage of 50x using the tool Filtlong. Adapters were removed with Porechop_ABI. The data was then assembled using Flye and the quality of the assemblies assessed using Quast, CheckM2 and Busco. Annotation of the antibiotic resistance (ABR) genes was done using AMRFinderPlus and ResFinder, plasmids were detected with MOB-suite and a phylogenetic analysis was performed using the k-mers based tool MashTree. In a previous experiment, the resistance phenotype was determined using EUVSEC3 plates.

Results and conclusion

The WGS analyses revealed a pattern of ABR genes – blaTEM-1B, sul1, dfrA14, tet(A) and parC and gyrA mutations – present in a large part of the SI strains (15 strains; 35.7%). Of these genes, sul1, dfrA14 and tet(A) were located on the pESI. blaTEM-1B, on the other hand, was located on an IncX4 plasmid frequently accompanying pESI (21 out of 32; 65.6%). A third plasmid, Incl-gamma/K1, did not appear to have a fixed ABR gene composition. In one strain it carried sul1 and in another strain the blaTEM-1B gene. The WGS data largely concords with the phenotypical data; the overall specificity and sensitivity of the WGS analysis compared to the phenotype were 95% and 90% respectively. A lacking expression, novel mechanisms or efflux pumps can be reasons for the observed discrepancies.

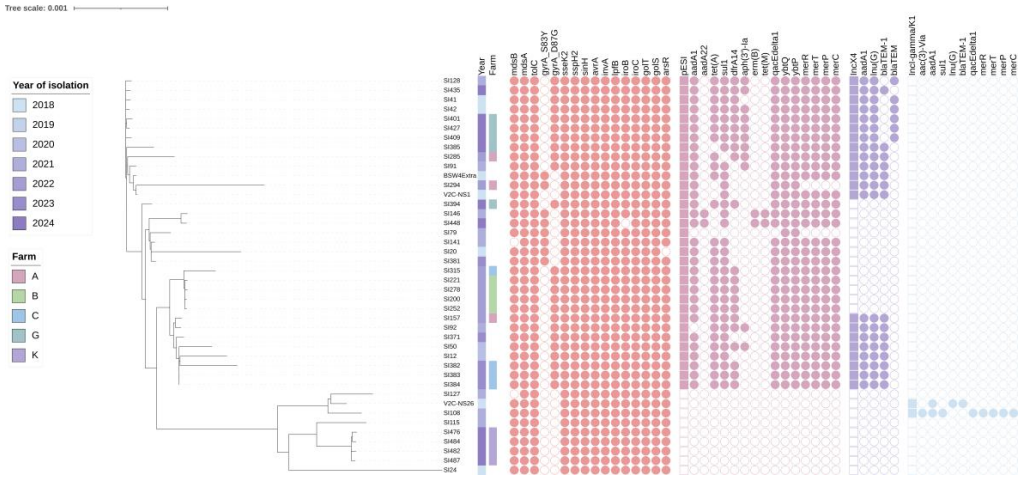

Figure 1: A phylogenetic tree based on the genomes of 42 Salmonella Infantis strains originating from the Belgian broiler sector. Year and farm of isolation are shown. Also shown are the antibiotic resistance genes as well as virulence and metal resistance genes obtained with AMRFinderPlus. In red are the chromosomal genes, in pink the genes on the pESI, purple the genes on an IncX4 plasmid and in light blue, genes on an Incl-gamma/K1 plasmid.

Targeting *Salmonella* Biofilm Formation in the Porcine Gut: Towards Alternative Antimicrobial Strategies to Reduce Persistence and AMR in Livestock

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Introduction

Chronic and asymptomatic *Salmonella* infections in pigs remain a major challenge for animal health, food safety, and antimicrobial resistance (AMR) control in livestock production. As pressure grows to reduce antibiotic use in animal husbandry, alternative antimicrobial strategies that target bacterial persistence mechanisms, such as biofilm formation, are gaining increasing interest. While biofilm formation is known to contribute to *Salmonella* survival outside the host, its role in persistence within the porcine gut remains poorly understood. This study therefore investigates the contribution of *in vivo* biofilm formation to *Salmonella* colonization and persistence in the porcine intestinal tract, including its interaction with the gut microbiota.

Materials and methods

The biofilm-forming capacity of *Salmonella* Typhimurium ATCC14028 was studied using the Simulator of the Mucosal Pig Intestinal Microbial Ecosystem (M-SPIME), an *in vitro* model that mimics the environmental conditions of the porcine gastrointestinal tract. To evaluate the role of biofilm formation, the wildtype strain was compared with biofilm-deficient deletion mutants. Initial single-compartment experiments demonstrated that the presence of mucus increased *Salmonella* cell densities, whereas the addition of gut microbiota reduced *Salmonella* abundance through microbial competition. Interestingly, mucus appeared to provide a protective niche for *Salmonella* in the presence of the microbiota. Although differences between strains were generally modest, deletion of the biofilm master regulator slightly reduced resistance to microbiota-mediated competition, while deletion of the two major biofilm matrix components resulted in a slightly increased competitive fitness.

Results and conclusion

Subsequently, a dynamic multi-compartment M-SPIME model was used to compare the wildtype strain with a biofilm-deficient mutant throughout the simulated gastrointestinal tract. Besides monitoring Salmonella cell counts, microbiome composition, mucus colonization, and chemical parameters were analyzed over time. Our results indicate that biofilm formation contributes to colonization of the ileal mucus layer, a region characterized by lower microbial diversity and potentially reduced colonization resistance. In addition, *Salmonella* infection altered both total microbial cell counts and microbiome composition, largely independent of biofilm formation capacity.

Overall, our findings suggest that mucus-associated biofilm formation may contribute to *Salmonella* persistence in the porcine gut. Targeting biofilm formation and mucus colonization could therefore represent a promising non-antibiotic strategy to reduce *Salmonella* carriage in pigs and help limit AMR selection pressure in livestock production systems. Future *in vivo* pig trials will further validate these observations.

Prophylactic antibiotic use in dog breeding: insights from a Belgian and Dutch breeder survey

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Introduction

Antibiotic use in dog breeding is common and is often driven by the perception that bacterial presence in the reproductive tract represents infection, despite the existence of a normal resident microbiota. Understanding current practices is crucial for guiding evidence-based recommendations and antimicrobial stewardship.

Materials and methods

A questionnaire on antibiotic use, bacteriology, and *Mycoplasma* testing was distributed to dog breeders in Belgium and the Netherlands. Complete responses were obtained from 420 breeders (23% Belgian, 77% Dutch). Associations between antibiotic use and breeder characteristics (country, experience, and number of litters per year) were analyzed using descriptive statistics, chi-square tests, Fisher's exact test, and logistic regression.

Results and conclusion

Overall, 32% of breeders reported having administered antibiotics to a bitch in the context of breeding, most commonly prior to mating (63%) or during lactation. The primary reasons were treatment of diagnosed infections (58%) and veterinary advice (36%), while prophylactic use was reported by 19%. Most treatments were short-term (<7 days, 54%), and all antibiotics were obtained from a veterinarian. Antibiotic use was higher among Belgian than Dutch breeders (41% vs 30%; $p = 0.039$). Breeder experience was significantly associated with antibiotic use, increasing from 15.8% in breeders with <5 years' experience to 40.8% in breeders with >20 years ($p = 0.0015$). Logistic regression confirmed a significant positive linear trend (Estimate = 0.873, $p = 0.00036$). Kennel size was not associated with antibiotic use ($p = 0.197$). Half of breeders who administered antibiotics to multiple bitches reported perceived improvements in reproductive outcomes.

Bacteriological screening was performed by 50% of breeders for bitches but only by 16% for males. *Mycoplasma* was known to 62% of breeders; however, knowledge regarding its role in subfertility was inconsistent, and 52% of breeders believed that a positive test result required antibiotic treatment prior to breeding.

Antibiotic use prior to mating is common among dog breeders, particularly among more experienced breeders and in Belgium. These practices appear influenced by long-standing breeding habits, veterinary advice, and interpretation of bacterial findings. The results highlight the need for targeted education and further research into the canine reproductive microbiome to support evidence-based breeding management and prudent antimicrobial use.

Cost analysis of the Belgian national antimicrobial resistance monitoring in livestock: effects on sampling design and statistical performance

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Introduction

As part of the European Union's harmonized monitoring framework, Belgium conducts antimicrobial resistance (AMR) monitoring in commensal bacteria from several livestock species and categories. The aim of this study was to conduct a cost analysis of the national AMR monitoring in livestock, and to explore sampling size scenarios in relation to their associated costs and statistical performance (power and confidence) of monitoring.

Materials and methods

Evaluation of the cost of the national AMR monitoring program was performed for poultry (broilers, laying and breeding hens), pigs (fattening) and cattle (veal calves and beef cattle), using unit cost aggregation. The various cost components were sampling, isolation, bacterial species identification (Maldi Tof) and AMR susceptibility testing (Sensititre).

The power and the confidence level of the AMR monitoring were also calculated for each animal category and for all animal categories per year, for various scenarios of sample sizes (i.e. number of isolates tested). A sensitivity analysis was conducted to assess the impact of potential variability in the estimated total monitoring costs.

Results and conclusion

The testing of the different sample size scenarios showed that if the sample size increases, the costs increase linearly. A sample size increase of 10 samples/isolates (e.g., from 170 to 180) can increase the yearly total costs per animal species by 5.2%.

The testing of the different scenarios further showed that if the sample size increases, the power and the confidence level also increase, providing a higher level of trust in the results of the monitoring program.

In the Belgian context, the total monitoring costs per animal category were estimated to be the highest for fattening pigs, broilers and veal calves (over 18% of total costs each, using 2024 data), while the estimated total cost for all animal species was T= 330,655 €.

Among the various monitoring activities, antimicrobial susceptibility testing emerged as the costliest component, representing 50.2% of the total monitoring costs.

The approach presented allows it to be used by other countries aiming to estimate the cost of their national AMR monitoring in animals or other similar activities. This economic and scenario testing analysis can be used to suggest informed decisions to improve AMR monitoring in animals and proper allocation of resources.

Enhancing One Health Through Wastewater-Based Surveillance: Development and Validation of a Multiplex ddPCR Tool to Differentiate Human and Livestock Contributions

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Introduction

Wastewater surveillance has emerged as valuable tool for public health, with notable successes in monitoring SARS-CoV-2 and, more recently, antimicrobial resistance (AMR) genes. Traditionally focused on human populations, this approach also holds significant promise for One Health surveillance. However, the extent to which livestock contributes to the microbial and genetic content detected in wastewater, compared to human sources, remains unclear. This gap in knowledge hampers our ability to normalize and accurately interpret wastewater surveillance data, particularly when integrating AMR gene signals.

Materials and methods

To address this, we developed and validated two duplex droplet digital PCR methods targeting fecal indicators specific to humans and key livestock species. These methods target cytochrome b of mitochondrial DNA specific to chickens and *Bacteroides* DNA specific either to humans, cows or pigs.

Results and conclusion

Method performance was evaluated against international standards to ensure reliability and harmonization across laboratories. A fecal DNA bank of 41 samples, collected from various human and animal sources, was used to retain only specific methods. Using serial dilutions of synthetic control plasmids carrying the targeted fecal indicator sequences, sensitivity was successfully assessed, with limits of detection below 10 copies. Applicability was demonstrated using wastewater samples spiked with livestock wastes. A one-year pilot monitoring was also conducted at two Belgian wastewater treatment plants, in regions with contrasting livestock populations. This study represents an essential step toward enhancing wastewater surveillance by integrating robust key species-specific fecal indicators. It lays the foundation for improved surveillance of livestock-associated outbreaks and the spread of AMR within a One Health framework.

Acknowledgments

This research was funded by the Belgian 'One Health' National Action Plan on the Fight Against Antimicrobial Resistance (2020–2024) and by internal Sciensano funding.

Genomic characterization of weaning-associated porcine *Escherichia coli* circulating in Belgium

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Introduction

Post-weaning *Escherichia coli* infections are a major cause of diarrhea, antimicrobial use, and economic losses in pig production. While classical enterotoxigenic *E. coli* (ETEC) carrying F4 or F18 fimbriae and enterotoxins remain the main causal agents in post-weaning diarrhea, recent studies suggest increasing genomic and virulence diversity among porcine *E. coli* populations. Characterizing circulating strains is essential for improving our understanding of pathogenic strain diversity and for supporting future antimicrobial alternative strategies.

Materials and methods

A collection of 198 *E. coli* isolates recovered from Belgian weaned pigs between 2021 and 2026 by ARSIA and DGZ was analyzed by whole-genome sequencing (Nanopore). After applying inclusion/exclusion criteria (weaned pigs, Belgian origin, intestinal isolates), 173 strains were retained. Virulence-associated genes and adhesins were identified using ABRicate 1.4.0 and Bakta 1.9.4. Serotyping and multilocus sequence typing (MLST) were performed using ECTyper 2.0.0 and PubMLST via Galaxy Europe. Pathotyping was performed by manual screening based on virulence profiles and clinical metadata.

Results and conclusion

Multiple virulence-associated profiles, adhesin combinations, serotypes, and MLST lineages were identified. ETEC was the predominant pathotype (55/173 isolates), with F18 more frequent than F4. Hybrid ETEC/STEC profiles were identified in 22/173 isolates. In addition to these classical profiles, several isolates displayed atypical, hybrid, or currently unclassified virulence-associated genomic profiles, highlighting the genomic diversity of circulating porcine E. coli populations, in which AIDA-I (Adhesin involved in diffuse adherence) was identified as the dominant adhesin among isolates lacking classical fimbriae. These findings provide an updated overview of the diversity of weaning-associated porcine *E. coli* in Belgium and emphasize the importance of considering this diversity when developing future antimicrobial alternative approaches, including phage-based strategies.

The research that yielded these results, was funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment through the contract RF 25/25 BATTLE – Bacteriophages and Tailocins Targeting and Lysing E. coli.

Development of an innovative monitoring protocol for *Enterococcus spp.* in broiler farms to support prudent antimicrobial use

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Introduction

Enterococcus spp. are part of the commensal gut microbiota of poultry but are also associated with clinical conditions such as arthritis and impaired growth in broiler production. These infections frequently result in increased antimicrobial use. In the context of antimicrobial resistance and current reduction targets, there is a clear need for a more targeted and preventive approach. However, a practical monitoring protocol to assess infection dynamics and underlying risk factors is currently lacking. The MonEntero project aims to develop such a protocol, incorporating both bacterial and viral co-infections.

Materials and methods

Within the MonEntero project, a multi-actor operational group including veterinarians, farm-advisors and broiler farmers was established. An extensive sampling protocol was implemented on six broiler farms, including scoring and the collection of enteric and other samples at multiple time points during the production cycle from both healthy and clinically affected animals. More detailed information on the protocol will be shared in future communications. Farms were selected based on historical clinical problems due to *E. cecorum*. An innovative nanopore sequencing-based diagnostic platform (PathoSense®) was used to enable the simultaneous identification of bacterial and viral agents without prior selection, in combination with classical diagnostic methods. In parallel, management practices and potential risk factors were documented through farm visits. Based on these data, a practical and economically feasible routine monitoring protocol is being developed and evaluated.

Results and conclusion

The project revealed interesting findings with respect to the presence of relevant viruses, bacteria and management related issues.

Nanopore-sequencing (Pathosense®) showed farm-specific evolutions and compositions of viral and bacterial load. Detailed analysis showed presence of Chicken astrovirus in all farms, Orthoreoviruses were also highly present and can be associated with locomotory problems in broilers. Intestinal integrity scoring showed that all investigated farms scored above average with respect to the Belgium reference values, suggesting that these farms were more susceptible for bacterial translocation from the intestine to extra-intestinal tissues. Throughout the project, special emphasis was placed on farm hygiene, particularly drinking water hygiene. Water samples of five out of six farms contained intestinal enterococci at the start of the production cycle, while two out of six farms showed overall poor water quality across multiple investigated parameters.

As a preliminary conclusion we can state that both the presence of the clinical agent and a possible predisposing factor, i.e. bacterial translocation from the intestine due to poor intestinal integrity, were present in the participating farms. As the agent was present in almost all farms drinking water samples, improving water hygiene- and disinfection management will be important to tackle *Enterococcus* spp. related problems.

Aeromonas spp. as bacterial candidate for antimicrobial resistance surveillance in aquatic animals in Belgium.

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Introduction

In Belgium, aquaculture is mostly semi-intensive, with salmonids raised in river-fed ponds. The absence of authorized fish-specific vaccines or antibiotics leads to the use of antimicrobials approved for other animal species. Unlike other food-producing animals, aquaculture lacks systematic monitoring of antimicrobial resistance (AMR). This study aimed to evaluate Aeromonas spp. as a potential AMR surveillance indicator by isolating them from fish skin mucus, water, and sediment across 22 Belgian fish farms during winter 2024-2025.

Materials and methods

Mucus swabs from fish skin (n=88), water (n=88), and sediment (n=29) samples were collected from 22 registered Belgian fish farms during winter 2024-2025. Bacteria were isolated on Glutamate Starch Phenol-red (GSP) Agar, incubated in parallel at 18°C and 30°C. Yellow colonies on GSP were subcultured and stored at -80°C. A selection of 35 colonies collected at 18°C were identified using API®20NE biochemical tests and 426 isolates were identified by MALDI-TOF mass spectrometry.

Results and conclusion

Suspected Aeromonas colonies were obtained on GSP, both at 18 and 30°C. Water samples yielded the highest number of isolates (n = 196 at 18 °C and n = 246 at 30 °C), followed by fish samples (n = 161 at 18 °C and n = 133 at 30 °C) and sediment samples (n = 69 at 18 °C and n = 81 at 30 °C), suggesting water as the most promising source for the isolation of *Aeromonas* spp. From the 35 selected isolates, to date, 19 isolates have been identified as *Aeromonas hydrophila/caviae* and 2 as *Aeromonas sobria* using the API® 20NE gallery. Of the 426 isolates, 171 were confirmed as *Aeromonas* spp., by MALDI-TOF MS. When considering sample type, *Aeromonas* spp. were identified in 101 out of 196 water isolates (51.5%), 37 out of 69 sediment isolates (53.6%), and 33 out of 161 fish mucus isolates (20,5%). Antimicrobial susceptibility testing is currently being performed using the EUAQUAN1 panel (ThermoFisher Scientific) to determine MICs for the selected isolates. The results will help assess the antimicrobial resistance patterns of *Aeromonas* spp. isolates in aquatic environments and contribute to a better understanding of their potential impact on aquatic ecosystem and public health.

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Isolation and characterization of bacteriophages targeting carbapenem-resistant *Klebsiella pneumoniae* from Tunisian hospital.

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Introduction

The global spread of carbapenem-resistant *Klebsiella pneumoniae* (CRKP), especially high-risk clones harboring carbapenemase genes, is a major public health concern because treatment options remain extremely limited. Bacteriophages are a promising alternative or adjunct to antibiotics against multidrug-resistant pathogens. This study aimed to characterize CRKP isolates circulating in a Tunisian hospital and to isolate and evaluate bacteriophages targeting the dominant capsular types associated with these strains.

Materials and methods

Between March and September 2024, 58 CRKP isolates were recovered from a hospital in Sousse, Tunisia. Antimicrobial susceptibility testing was performed according to CLSI guidelines, followed by whole genome sequencing using Illumina technology. Resistance genes, plasmid content, sequence types, and capsular loci were analyzed bioinformatically. Eight wastewater samples collected from hospital effluents and wastewater treatment plants were screened for bacteriophages using enrichment methods. Isolated phages were purified, amplified, and characterized by host range analysis, efficiency of plating (EOP), adsorption kinetics, thermal and pH stability assays, genomic analysis, in vitro bacterial growth inhibition assays, and *Galleria mellonella* infection models.

Results and conclusion

The CRKP population was dominated by the high-risk ST147 clone carrying blaNDM-5, blaOXA-48, and blaCTX-M-15 genes associated with IncF and IncL plasmids. The predominant capsular types were KL51 and KL64. Thirteen bacteriophages targeting eight different capsular types were successfully isolated, with titers ranging from 2.3×10^7 to 2.8×10^9 PFU/mL. Most phages exhibited high lytic activity with EOP values ≥ 0.5 and rapid adsorption kinetics, achieving ≥90% adsorption within ≤10 minutes. Genomic analysis identified nine obligately lytic phages lacking lysogeny-associated genes. Several phages demonstrated strong thermal and pH stability as well as significant in vitro growth inhibition of CRKP strains. In the *Galleria mellonella* model, phages targeting KL51 and KL136 significantly improved larval survival, reaching up to 86.7% survival compared to infected untreated controls. These findings highlight the therapeutic potential of locally isolated bacteriophages against epidemic CRKP clones and support the development of personalized phage cocktails targeting dominant capsular types circulating in Tunisian hospitals.

Spreading more than nutrients: does fertilization with manure increases antibiotics and antibiotic-resistance genes in soils?

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Introduction

Antibiotic resistance is an escalating global health crisis escalated by the over- and misuse of antibiotics in healthcare and agriculture, which can introduce both antibiotic residues and resistant bacteria in the environment. Even low concentrations can promote the emergence and persistence of resistance by exerting selective pressure on bacteria. Resistance genes can be exchanged between bacteria of different ecological niches, enabling antibiotic resistance to transcend geographical and national boundaries. The interconnection of human, animal and environmental health, described as the "One Health" approach, highlights the necessity to address antibiotic resistance in environmental systems. This includes understanding the transmission of residues, resistance genes and resistant bacteria across humans, animals, and environmental compartments such as soil, water, and food. This study, conducted within the Nutritive (https://nutritive.es/) framework, aims to generate evidence on the occurrence of these pollutants in manure and their subsequent transfer to soil following fertilization.

Materials and methods

A total of 30 case study farms across seven countries (Belgium, The Netherlands, Germany, Ireland, Italy, Spain and Poland) were sampled. For each case study, soil samples were collected immediately before fertilization, directly after fertilization, and again three weeks later. The manure applied to the fields was also sampled, along with a repeat sample collected six months later. Antibiotic resistant indicator organisms, extended-spectrum β-lactamase producing *Escherichia coli* and vancomycin-resistant *Enterococci* (VRE), were targeted in manure and soil samples. Antibiotic residues were quantified using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) applying a 63 multi-residue method. In addition, 36 antibiotic-resistance genes will be quantified by qPCR in each sample.

Results and conclusion

Not all samples have been analyzed yet. Until now, antibiotic residues were detected in 75% of the manure samples analyzed (n = 31), with up to six different residues identified in a single sample. Doxycycline, lincomycin and oxytetracycline were the most prevalent compounds, and the highest concentration (3600 µg/kg lincomycin) was measured in Italian pig manure. Concentrations in manure were higher than quantified in soil samples. Following fertilization, no significant differences were observed in antibiotic residue concentrations or in the number of residues per sample between soils collected before and after fertilization (n = 80). Fertilization did not significantly increase antibiotic residue levels in the soil, suggesting that these residues were already present and may persist in soils independently of recent fertilization. The concentration and dynamics of antibiotic residues varied considerably depending on the specific case study and the physico-chemical properties of the compounds. Among the 20 antibiotics detected in soil, oxytetracycline, flumequine, and doxycycline were the most frequently observed. Chlortetracycline exhibited the highest post-fertilization concentration, reaching 1305 µg/kg.

Regarding antibiotic resistant bacteria, the manure samples contained ESBL *E. coli* (13 %) and VRE (68 %). Following fertilization, soil samples showed an increase in E. coli (+31 %), ESBL-producing *E. coli* (+8 %), and total *Enterococci* (+31 %). The prevalence of VRE remained unchanged. After three weeks, total *E. coli* counts remained stable, while ESBL-producing *E. coli* and total *enterococci* declined to levels observed before fertilization. To conclude, manure introduced microbial and chemical contaminants to the soil. Results of antibiotic resistance genes will be available by the time of the symposium, when further updated findings will also be presented.

The spread of antibiotic resistance in the environment: A study of the effect of treatments applied to sewage sludge used as a soil amendment on antibiotic-resistant bacteria and resistance genes

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Introduction

Wastewater treatment plants (WWTPs) receive and treat thousands of cubic metres of water every day, containing antibiotic-resistant bacteria (ARBs) and resistance genes (ARGs), making these WWTPs 'hotspots' for antibiotic resistance. The main waste produced by these processes is sewage sludge, resulting from the biological activity of the living microorganisms in the plants that break down the matter carried by the wastewater. The "Antibioboue" project has the objective to study the antibiotic resistance dissemination in the environment, as some of this sludge is used in agriculture via land application.

Materials and methods

Seven WWTPs and one biocentre were sampled (18 samples in total) to assess the abundance and fate of bacteria (*E. coli*, *E. coli* ESBL, *enterococci* and total bacteria) and genes (16S rDNA, intl1, blaCTXM1, vanA, ermB, aadA1, sul1, blaKPC and tetW) across different treatment processes (liming, dewatering, composting). Gene quantification was performed by qPCR.

Results and conclusion

The relative abundance of total bacteria ranges from 10^3 to 10^7 CFU/g wet sludge in untreated sludge and is reduced by 1 to 2 log by treatment. No *E. coli* ESBL was detected in the sludge treated. The detected ARG levels vary widely, ranging from 10^6 (*VanA*, limed sludge) to 10^{11} gene copies/g DM (*intl1*, raw sludge). The *intl1*, *ermB* and *sul1* genes are, in most cases, the most abundant. The $bla_{CTX\text{-}M\text{-}1}$, bla_{KPC} and *VanA* genes were detected less frequently (below the LOQ). Liming, which is the most commonly used treatment in Wallonia, achieved the best reduction rates.

This project highlights the presence of resistance genes in the sludge treatment process and the importance of monitoring effluent flows from the wastewater treatment plant into the soil, with the aim, firstly, of studying the dynamics of antibiotic resistance spread and, secondly, of assessing the associated environmental and health risks.

Assessing representativeness and statistical trend analysis in Belgian livestock AMR monitoring

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Introduction

Antimicrobial resistance (AMR) is a major threat to both human and animal health. In the European Union, harmonized monitoring of AMR in food-producing animals is mandatory within a One Health framework. Since 2011, Belgium has conducted annual monitoring of commensal *Escherichia coli* in livestock. This study aimed to evaluate the Belgian official AMR monitoring system, with a focus on sampling representativeness and statistical approaches used for national AMR trend analyses.

Materials and methods

Data from commensal *E. coli* collected between 2017 and 2019 in veal calves, broiler chickens, fattening pigs, and beef cattle were analysed. Sampling representativeness was assessed through evaluation of epidemiological units, temporal distribution of sampling, slaughterhouse coverage, and geographical clustering using SaTScan™. Several statistical approaches for AMR trend analyses were compared, including univariate logistic regression, generalized estimating equations (GEE), mixed-effects logistic regression, and count-based regression models.

Results and conclusion

The monitoring system generally achieved the slaughterhouse coverage and target sample sizes required under European legislation. However, repeated sampling of the same herds was observed across all monitored species, both within the same day and within the same year. Significant non-uniform temporal sampling distributions and statistically significant geographical clusters were identified in all species and years evaluated. Among the statistical approaches assessed, multivariate logistic GEE models appeared particularly suitable for national AMR trend analyses, as they account for correlations between isolates while remaining computationally efficient for routine surveillance.

Overall, the Belgian AMR monitoring system provides valuable long-term surveillance data for livestock AMR surveillance. However, several aspects related to sampling representativeness were identified and should be considered to further optimize the monitoring system and support future methodological discussions at the European level.

Characterization and efficacy evaluation in the *Galleria mellonella* model of four novel bacteriophages targeting *Aeromonas salmonicida*

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Introduction

The Gram-negative bacterium *Aeromonas salmonicida* subsp. *salmonicida* is a major fish pathogen causing furunculosis, one of the main bacterial diseases affecting salmonids. Since this bacterium leads to significant losses in aquaculture worldwide and frequently displays antimicrobial resistance, this study aimed to isolate bacteriophages targeting *A. salmonicida*, characterize them in vitro, and evaluate their therapeutic potential using the *Galleria mellonella* larvae model.

Materials and methods

Phages were isolated from fish farms and aquatic environments. Phage morphology, host range and biochemical stability were determined, and genomes were sequenced. Their therapeutic efficacy was then evaluated in infected *G. mellonella* larvae.

Results and conclusion

Four novel phages presenting a myovirus morphotype were isolated. Three belonged to the *Straboviridae* family, while one remained unclassified. Three of the phages were highly stable and active against other *A. salmonicida*, including multidrug-resistant isolates. Single phage administrations prolonged the survival of infected *G. mellonella* but did not fully eradicate the bacteria.

The *in vitro* results support the potential of these bacteriophages as alternatives to antibiotics against *A. salmonicida* infection in fish. Although incomplete bacterial clearance was observed in *G. mellonella*, improved survival highlights their therapeutic relevance. Further optimization of treatment strategies or phage combinations may enhance efficacy in aquaculture applications.