



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.

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| 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 |
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| 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) |
| 11h45 | Antibiotic resistance in aquatic environmental hotspots (Laurens Tuts, ILVO) |
| 12h | Poster prizes announcement and closing remarks by Prof Damien Thiry |
| 12h15 | Lunch and poster session |

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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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Country: Switzerland

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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.

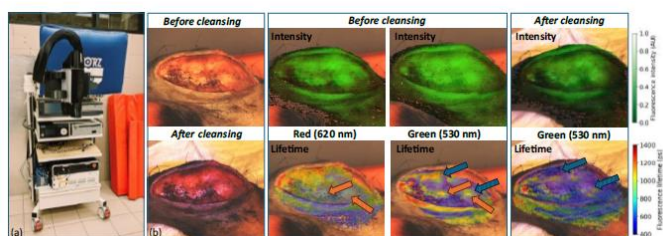


Fig. 1 (a) tauCAM system (b) FI and FL images of a deep wound on the right hind leg of a female dog, 3 days after being hit by a car, before and after cleansing (i.e., saline) and sharp debridement. Arrows: FL differences before and after cleansing (blue), and between spectral bands (orange).

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, IncI-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 concurs 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.

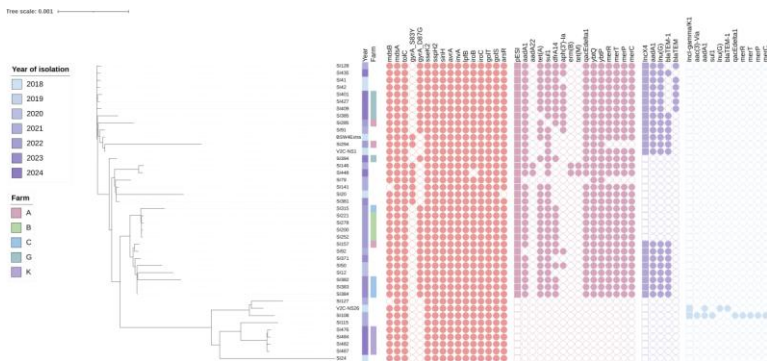


Figure 1: A phylogenetic tree based on the genomes of 42 *Salmonella* Infantis strains originating from the Belgian broiler sector. Year and farm of isolation are shown. Also shown are the antibiotic resistance genes as well as virulence and metal resistance genes obtained with AMRFinderPlus. In red 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 IncI-gamma/K1 plasmid.

Outpatient Antibiotic Consumption Trends in Belgium: A Comparative Analysis of Reimbursement and Sales Data, 2013-2022

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Introduction

Antimicrobial resistance (AMR) is a global public health concern, necessitating close and timely monitoring of antibiotic consumption (AMC). In Belgium, AMC surveillance traditionally relies on reimbursement data, excluding over-the-counter non-reimbursed or imported products and involving a time lag. This study investigates disparities in AMC between reimbursement data and retail data, providing insights into AMC variations. Additionally this study seeks to critically evaluate the validity and representativeness of the reimbursed data in accurately reflecting the true extent of AMC in the country.

Materials and methods

Utilizing reimbursement data from the National Institute for Health and Disability Insurance (NIHDI) and retail data (IQVIA Sales data; www.iqvia.com) for systemic antibacterials (ATC Group J01), outpatient consumption was estimated for the period 2013-2022. Volume of antimicrobials was measured in Defined Daily Doses (DDDs - WHO ATC/DDD Index 2023), while population data were extracted from Eurostat. Relative differences (RDs) in DDDs per 1000 inhabitants per day (DID) were computed, and validated through correlation analysis (Pearson's r) and Bland-Altman plots.

Results and conclusion

J01 antibacterial sales declined from 23.10 DID (2013) to 20.85 (2022). Non-linear decreases, notably during the Covid-19 pandemic (21.54 DID in 2019 to 16.69 in 2020), followed by a rebound to pre-pandemic quantities in 2022 were observed (Figure 1). Reimbursement NIHDI data slightly underestimated IQVIA sales, with RDs ranging from 2% (2013) to 9% (2022). Notable differences, especially in recent years were attributed to quinolone reimbursement criteria changes implemented by law in Belgium in 2018, reducing the reimbursed proportion from 99% (2017) to 35% (2022). ATC-3 level analysis revealed disparities in low-DID groups (J01B, J01E and J01G). Notably, a small proportion of amphenicols (J01B) were reimbursed (<10%), with fluimucil frequently bought and remaining unreimbursed. Overall and across ATC3 groups, the correlation between NIHDI and IQVIA estimates was almost perfect across years and the Bland-Altman plots showed high agreement.

Conclusion

Reimbursement data are reliable for outpatient AMC monitoring with slightly lower estimates than retail data across most categories. The 2018 quinolone reimbursement criteria change highlights the necessity of incorporating retail data for accurate assessments in this specific category. The synergistic use of reimbursement and retail datasets is crucial for a comprehensive understanding of consumption patterns, supporting effective AMR mitigation strategies in Belgium.

Met opmerkingen [ML1]: Vanaf hier verder doen

Assessment of environmental reservoirs for acquired antibiotic resistance determinants in Hungary from a One Health perspective

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*The two authors contributed equally.

Introduction

The widespread use of antibiotics as a selection factor led to the emergence and dissemination of antibiotic-resistant bacteria worldwide, contributing also to global food safety risks. The medical and agricultural use of antibiotics provides a selection advantage for the development and spread of antibiotic resistant bacteria not only in livestock, but also in the microflora of the soil and in various environmental reservoirs, by entering the soil for example through manure or sewage sludge application and potentially also by irrigation. The aim of our studies is to analyse possible epidemiological interactions between antibiotic-resistant bacteria of food animal, wild animal and/or environmental origin from a One Health perspective, and to explore possible routes of dissemination for acquired antibiotic resistance genes (ARGs) and resistant bacterial strains.

Materials and methods

Escherichia coli and/or *Pseudomonas* spp. isolates were cultured on antibiotic containing screen agar plates from various specimens (faeces, nose swab and/or oral swab) of domestic pig and wild boar (*Sus scrofa*), hunting dog (*Canis lupus familiaris*), fallow deer (*Dama dama*) and from environmental samples (such as street drain water and puddle water) in Hungary, and submitted for draft genome sequencing on Illumina platform. Shotgun metagenomic sequencing of gDNA purified from farmed African catfish hybrid (*Clarias gariepinus* x *Heterobranchus longifilis*) intestinal content sample was performed on Illumina NovaSeq 6000 platform using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina by iBioscience Ltd. (Pécs, Hungary). Contigs assembled from NGS sequencing data were analysed by the ABRicate version 1.0.1 tool against the ResFinder reference database to detect acquired ARGs. The cultured antibiotic-resistant isolates were further characterized by molecular and classical microbiological methods.

Results and conclusion

The assembled intestinal metagenomic contigs of the farmed African catfish carried acquired ARGs of the antibiotic classes tetracyclines, macrolides, aminoglycosides, quinolones, sulphonamides and others. To the best of our knowledge, the acquired resistome of farmed African catfish has not yet been examined in Hungary by metagenomic methods. In addition, several of the acquired ARGs identified in the catfish intestinal microbiome in this study have also been reported from farmed catfish by other international studies. Bioinformatic analyses of the draft genome sequenced *E. coli* and *Pseudomonas*

spp. isolates revealed a variety of acquired ARGs, including a *P. aeruginosa* isolate that belongs to the epidemic high-risk clone ST111. This suggests that environmental reservoirs can also be involved in its dissemination. This work was supported by the RRF-2.3.1-21-2022-00007 Hungarian National Laboratory project and by the NeoGiant Horizon 2020 project (Grant agreement ID 101036768).

Isolation, *in vitro* characterization and efficacy assessment in *Galleria mellonella* larvae of four bacteriophages targeting *Aeromonas salmonicida*

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⁵ Fish pathology laboratory, CER groupe, Marche-en-Famenne, Belgium

Introduction

The Gram-negative bacterium *Aeromonas* (*A.*) *salmonicida* is a primary fish pathogen that causes furunculosis in salmonids as well as septicemia in a variety of fish. In one hand because this disease is responsible for significant losses in salmonid production worldwide and in the other hand because of the frightening tendency of this bacteria to exhibit antimicrobial (multi-)resistances, phage therapy could represent a leading alternative to treat this infection in aquaculture. The aims of this study were to create a collection of *A. salmonicida* strains, isolate phages targeting these strains, phenotypically and genomically characterize these newly isolated phages and assess their potential for phage therapy in a preliminary *in vivo* model.

Materials and methods

An ATCC and three field strains of *A. salmonicida* isolated in Belgium were used for phage isolation. Different water sources were collected in Belgium and France to isolate *A. salmonicida*-specific phages: wastewater, fish farming water and natural aquatic environment water. Newly isolated phages were characterized *in vitro* for their ability to infect other *A. salmonicida* strains and their stability at different temperatures (25°C, 37°C, 45°C and 60°C) and units of pH (2, 4, 6, 8, 10, 12). Phage morphologies and genomes were investigated by electron microscopy and Illumina sequencing. The safety and efficacy of these phages were finally assessed at two treatment doses (MOI 10 and 100) during four days in a preliminary *in vivo* model using *Galleria* (*G.*) *mellonella* larvae.

Results and conclusion

Four new phages active against *A. salmonicida*, named vB_AsaM_ULASA1 (47,813bp), vB_AsaM_ULASA2 (170,823bp), vB_AsaM_ULASA3 (164,381bp) and vB_AsaM_ULASA4 (171,205bp), were isolated from water samples collected in fish farms and natural aquatic environments. Phages ULASA2, 3 and 4 were active against other *A. salmonicida* strains and showed a high resistance to temperatures and pH tested while the phage ULASA1 showed a more restricted host spectrum and less biochemical tolerance. Genomic analysis showed that phages ULASA2, 3 and 4 belong to the *Straboviridae* family but no family has already been attributed to the phage ULASA1. All four presented a myovirus morphotype. Four-day efficacy experiments in the preliminary *in vivo* model of *G. mellonella* larvae showed that phages ULASA1, 2 and 4 were responsible for a significant extension in the larval survival time at the two treatment doses tested (MOI 10 and 100) while the phage ULASA3 only showed a significant effect at MOI 100. In light of these results, these phages targeting *A. salmonicida* could represent potential new candidates for the development of anti-furunculosis phage treatments in aquaculture.

Screening for carbapenemase-producing *E. coli* and *K. pneumoniae* in freshwater, bathing water and hospital continuums, and determination of the carbapenemases (*bla_{NDM}*, *bla_{KPC}*, *bla_{OXA-48}*, *bla_{VIM}*, *bla_{IMP}*) by antibiogram, real-time PCR, immunochromatographic tests and whole genome sequencing

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Introduction

Antimicrobial resistance (AMR) is recognized by the WHO as one of the greatest threats to global health. Carbapenem resistance is a major concern, as this class of antibiotics, belonging to the β -lactam family, is used as a last resort molecule in hospitals in human medicine. Enteric bacteria, including *E. coli* and *K. pneumoniae*, from humans and animals can be released in aquatic environment and be a vector for AMR.

Materials and methods

This study focused on carbapenemase (CPE)-producing *E. coli* and *K. pneumoniae* in the aquatic environment, based on the screening of 3 hospital continuums (hospital-WWTP-receiving river), 20 surface waters and 29 bathing waters in 2022. Phenotypic tests including disk combination tests to identify the Ambler class, genotypic tests (real-time PCR) and immunochromatographic tests were used to characterize each isolate and determine the carbapenemase produced (*bla_{NDM}*, *bla_{KPC}*, *bla_{OXA-48}*, *bla_{VIM}*, *bla_{IMP}*). The genomes of 11 *E. coli* CPE and 15 *K. pneumoniae* CPE were sequenced.

Results and conclusion

No *E. coli* nor *K. pneumoniae* CPE was isolated from bathing waters, while 24 *E. coli* CPE and 27 *K. pneumoniae* CPE were isolated from 6 of the 20 surface water samples and from hospital continuums. Of the 24 *E. coli* CPE strains, 20 had the *bla_{KPC}* gene (83.3%) and 4 the *bla_{OXA}* gene (16.6%), while of the 27 *K. pneumoniae* CPE strains, the number of strains with CPE genes was: 11 *bla_{KPC}* (40.7%), 9 *bla_{OXA}* (33.3%), 1 *bla_{VIM}* (3.7%), 1 *bla_{NDM}* (3.7%) and 5 (18.5%) were unidentified. The genomes of 11 *E. coli* CPE and 15 *K. pneumoniae* CPE were sequenced, and all possessed CPE genes - *bla_{KPC-3}* (n=14), *bla_{OXA-48}* (n=9), *bla_{OXA-244}* (n=1), *bla_{VIM-1}* (n=1) and *bla_{NDM-5}* (n=1) - mostly coupled to ESBL-encoding genes. This study demonstrated that *E. coli* and *K. pneumoniae* CPE are disseminated in the Belgian aquatic environment.

Exposure risk of the Belgian human population to antimicrobial-resistant bacteria originating from pet cats and dogs

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Introduction

The possibility of pet cats and dogs acting as reservoirs for antimicrobial-resistant bacteria poses a serious threat to public health, given the importance of antimicrobials in modern medicine, the growing population of pets in Belgium, and the lack of both data gathering and sensitization of pet owners and veterinarians regarding antimicrobials usage. Pet cats and dogs can become infected by resistant bacteria from various sources, including their environment, food, other animals, or even humans. Furthermore, their direct or indirect consumption of antimicrobials can stimulate the selection of resistance in both commensal and pathogenic bacteria.

It is therefore important to identify the pathways of antimicrobial-resistant bacteria transmission between pet cats/dogs, close-contact humans such as pet owners or veterinarians, and the general population in Belgium. It is also crucial to estimate the risk for a human to be exposed to resistant bacteria originating from pets, based on those pathways and on the available data. Finally, this estimation allows the highlighting of the most effective ways to mitigate the risk.

Materials and methods

To achieve this, we are developing a probabilistic quantitative risk assessment model and testing various “what-if” scenarios. This model integrates data coming from various samplings analysis, public surveys, other scientific studies and experts opinions through a Monte-Carlo algorithm to compute an estimate of the risk. Then, different scenarios can be tested by modifying the parameters of the model and observing the variations of this estimate.

Results and conclusion

The model is divided into three modules, each representing transmission between pets and close-contact humans, pets and the general population, and pets and the general population. The pathways identified include meal preparation, feces/urine, blood, surfaces, hair/dead skin, physical interactions, biting/scratching, living carriers, airborne transmission and vertical transmission. The populations and the pathways linking them together are represented by estimations, formulas and probability distributions. At present, we do not yet have results from the risk analysis.

Evaluation of a Novel Herbal Supplement for Prevention of Neonatal Calf Pathologies: A Consolidated Study

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Introduction

Neonatal conditions such as diarrhea and bovine respiratory diseases contribute significantly to illness and death in calves prior to weaning. These health issues are typically multifactorial, resulting from a mix of stress and weakened immunity that enables various pathogens to emerge. Primary treatment strategies for digestive and respiratory pathologies often include antibiotics and anti-inflammatory drugs. However, the growing importance of preventive measures is being recognized due to concerns around antimicrobial resistance, animal health, and welfare. Plant-based formulations present a promising yet underutilized potential solution as an alternative or supplementary preventive treatment in farm settings. With this in mind, the primary goal of this research was to evaluate the impact of administering a novel preventive herbal supplement on the prevalence of calf diseases, antibiotic usage, as well as performance and health indicators.

Materials and methods

A total of 132 calves from 6 Walloon farms, originating from 2 consolidated studies, were involved in the study. Half of the calves received a placebo, and the other half received an herbal supplement administered in feed in powder form from day 1 after colostrum feeding until day 10. Digestive and respiratory disease prevalence, as well as curative treatment prevalence, were recorded until 28 days of age. Performance and health parameters were also recorded until weaning (e.g., serum albumin at day 28 and weaning; age, weight, and average daily gain at weaning; death status). Differences according to the immune transfer level were also investigated. Generalized linear models were used, including the effects of treatment, farm, as well as the interaction between treatment and farm as fixed effects. A multiple comparison test was conducted to assess the existence of a significant difference between the treatment group and the placebo group.

Results and conclusion

A trend was observed for reduced antibiotic and anti-*Cryptosporidium* treatment usage (p-value = 0.12) among calves given the herbal supplement (36% of treated calves vs. 48% of placebo calves received at least one treatment). Calves receiving the herbal supplement also exhibited a trend towards decreased pathology prevalence, particularly digestive pathologies (50% of treated calves experienced diarrhea compared to 62% of placebo calves), and higher serum albumin content at day 28 (p-value = 0.08). Calves with deficient immunity transfer appeared to be more responsive to the treatment in terms of digestive pathologies. No significant differences were observed in other tested parameters between the placebo and treated groups. The results of this consolidated study are promising, and additional field trials are planned to further investigate the potential of this new supplement for disease prevention in calves.

A Novel Consolidated Blood Biomarker Index for Enhanced Health Monitoring, Welfare, and Antibiotic Reduction in Fresh Cows

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Introduction

The dairy industry is under increasing pressure to reduce antibiotic use while ensuring optimal health and welfare for dairy cattle. Early detection of health issues in dairy cows, particularly within the first 0-60 days post-calving, is crucial for effective intervention strategies and improving welfare. Blood biomarkers offer a promising avenue for assessing the health status of cows, providing valuable insights into nutritional status, inflammation, and metabolic health. Developing reliable and easy-to-use indicators that can accurately identify cows at risk of health complications is useful for proactive management and antibiotic reduction efforts. In this context, this study aims to introduce an individual health index, primarily suited for fresh cows, utilizing a panel of blood biomarkers including albumin, urea, cholesterol, NEFA (non-esterified fatty acids), globulin, and the albumin/globulin ratio.

Materials and methods

The consolidated index for each animal was calculated using a formula based on the albumin value, with a scale of 1 to 10 (1 point for albumin ≤ 25 g/L to 10 points for albumin ≥ 34 g/L). A penalty or bonus was added to the score based on the values of other blood biomarkers (urea, cholesterol, NEFA, globulin, albumin/globulin ratio). The thresholds used in the formula were derived from veterinary experts' practical experience and scientific literature. Data for the development of the index were collected from a sample of 260 animals with varying days in milk (1 to 400 days). Descriptive statistics were performed to assess the distribution and relevance of the index.

Results and conclusion

The distribution of the index value calculated from 260 animals revealed an average value of 8.34 and a standard deviation of 4.89. Significant differences were observed between farms, aligning with management practices observed in the field. Upon expert consultation, it was confirmed that the index is most suitable for fresh cows (0-60 days in milk). Fresh cows with an index value of 0 or lower were identified as being at high risk of health issues, cows with an index value between 0 and 6 were considered suboptimal, and cows with an index value above 6 were deemed at low risk of health issues. In conclusion, the novel consolidated blood biomarker index, validated by expert consultation, serves as a valuable tool for assessing cow health, particularly for fresh cows. This could facilitate early intervention, thereby enhancing welfare and decreasing antibiotic use in the dairy industry.

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Exploring Factors Influencing Serum Selenium Levels in Adult Cows: Implications for Livestock Health Management and Antibiotic Reduction

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Introduction

Optimizing nutrition in livestock management is pivotal for animal health and productivity, with selenium (Se) playing a crucial role in physiological processes. This study investigates serum Se levels in adult cows, focusing on beef and dairy cattle in the Walloon region of Belgium and adjacent areas. By analyzing a significant dataset of individual serum analyses, we aim to identify factors influencing serum Se concentrations, including temporal and regional variations. Understanding these dynamics is essential for developing targeted nutritional interventions and establishing effective strategies for maintaining optimal Se nutrition in cattle herds. Monitoring Se levels in cattle is crucial for antibiotic reduction efforts as it can provide insights into potential opportunities for preventive nutritional interventions, ultimately promoting healthier immune function and potentially decreasing the reliance on antibiotics in livestock management.

Materials and methods

We compiled a comprehensive dataset from 1280 serum analyses collected from dairy and beef cows in various regions of Wallonia between January 2020 and June 2023. Serum Se levels were measured by Synlab Belgium SRL using atomic absorption spectroscopy. The dataset underwent cleansing for reliability. To ensure representativeness, we aggregated the data by calculating the average serum Se concentration for each unique combination of postal code and date, resulting in an aggregated dataset of 947 observations. Generalized linear models (GLMs) were then used to assess factors influencing serum Se levels, including region, year, trimester, season, laboratory site, and their intricate interactions. Visualizations included maps of Se concentrations by region and plots of Se fluctuations over time.

Results and conclusion

GLMs and visualizations unveiled variations in serum Se concentrations among different provinces, highlighting disparities in Se availability across regions. Notably, our analysis indicated a significantly higher average Se concentration in the Hainaut province compared to Liège and Luxembourg. Temporal analysis revealed fluctuations in Se levels over different timeframes. For example, the 2021-2022 winter period coincided with a notable increase in Se deficiency among cattle, affecting more than 30% of animals. These findings emphasize the dynamic nature of serum Se concentrations and the need for continuous monitoring to detect and address fluctuations in Se status promptly. In conclusion, our study presents useful insights into the intricate dynamics of serum Se concentrations in adult cows in Wallonia and neighboring regions. By shedding light on regional disparities and temporal fluctuations in Se levels, our research highlights the importance of implementing proactive measures, such as selenium supplementation, to mitigate nutritional deficiencies and promote overall herd health. Furthermore, our findings underscore the potential role of selenium monitoring in reducing antibiotic use by addressing underlying nutritional imbalances that may contribute to susceptibility to infections. Moving forward, our study sets the foundation for the creation of a long-term observatory of serum Se in cattle, facilitating continuous monitoring and informed nutritional adjustments to improve cattle health and production efficiency while simultaneously contributing to antibiotic stewardship efforts in livestock management.

Antimicrobial resistance of *Streptococcus suis* isolated from swine streptococcosis in Brazil

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Introduction

Streptococcus suis (*S. suis*) is a gram-positive bacterium that causes swine streptococcosis and is considered an emerging zoonosis. In pigs, the disease can manifest in different clinical forms, such as meningitis, polyarthritis, endocarditis, pneumonia, septicemia, and polyserositis. Additionally, some strains of *S. suis* can be pathogenic to other species such as cattle, poultry, and humans. Meningitis is the most common form of the disease in humans, primarily affecting swine farmers, slaughterhouse workers, and veterinarians, and can also be transmitted through contaminated meat. Several classes of antimicrobials are commonly used in veterinary medicine to treat *S. suis* infection. In recent years, an increase in reports of resistance among *S. suis* strains in animal treatment has been observed. Therefore, the objective of this study was to investigate the antimicrobial resistance profile of *S. suis* isolated from different clinical conditions in swine in Brazil.

Materials and methods

During 2020 to 2023, clinical samples originating from swabs and/or organs collected from pigs presenting lesions of pneumonia, meningitis, polyarthritis, and septicemia were subjected to bacterial isolation at the VERTÀ laboratory. *Streptococcus suis* identification was confirmed through Polymerase Chain Reaction (PCR). Antimicrobial susceptibility testing was conducted using the disk diffusion method as recommended by The Clinical & Laboratory Standards Institute (CLSI), against a panel of 24 antimicrobials.

Results and conclusion

In total, 327 isolates were molecularly confirmed as *S. suis*, comprising clinical presentations of pneumonia (127/327 – 38.8%), meningitis (102/327 – 31.2%), polyarthritis (71/327 – 21.7%), and septicemia (27/327 – 8.3%). Table 1 describes the percentage of antimicrobial sensitivity. A multidrug-resistant profile was observed for several tested antimicrobials. Tetracycline, lincomycin, and neomycin exhibited the highest resistance rates, while amoxicillin + clavulanic acid, amoxicillin, and florfenicol showed the highest antimicrobial sensitivity rates. The use of bacterial isolation followed by antimicrobial sensitivity testing is crucial for devising effective strategies to control *S. suis* infections.

Table 1. Antibiotic resistance profile of 327 isolates of *Streptococcus suis* from swine with different clinical conditions.

Antimicrobial	Sensitivity % (N)	Antimicrobial	Sensitivity % (N)
Amoxicillin + Clavulanate	98,5 (322)	Norfloxacin	59,0 (193)
Amoxicillin	97,0 (317)	Spectinomycin	54,7 (179)
Florfenicol	95,4 (312)	Ciprofloxacin	51,8 (169)
Fosfomycin	90,9 (297)	Enrofloxacin	47,7 (156)
Ceftiofur	89,9 (294)	Gentamicin	46,5 (152)
Doxycycline	89,9 (294)	Sulfa + Trimethoprim	46,2 (151)
Ampicillin	87,5 (286)	Streptomycin	45,9 (150)
Cephalothin	85,7 (280)	Penicillin	43,7 (143)
Erythromycin	84,7 (277)	Neomycin	29,4 (96)
Marbofloxacin	75,0 (245)	Lincomycin	25,0 (81)

Lincomycin + Spectinomycin	68,8 (225)	Tetracycline	11,9 (39)
Oxacillin	60,0 (196)	Cephalexin	11,0 (36)