“Antimicrobial resistance: a challenge for public health, animal health and the environment”

Scientific symposium

June 25, 2024

Brussels – webinar

Program

9h30 Welcome words by Prof. Dr. Dominiek Maes – Ghent University and chair of AMCRA

9h35 Keynote presentation by Dr. Jean-Yves Madec (ANSES, France): How to monitor AMR in diseased animals ? The example of the French Resapath network.

10h00 First session chaired by Prof. Dr. Dominiek Maes – Ghent University and chair of AMCRA

10h05 Pulmonary Aspergillosis in Humboldt Penguins—Susceptibility patterns and molecular epidemiology of clinical and environmental Aspergillus fumigatus isolates from a Belgian zoo, 2017–2022 – Hanne Debergh (Sciensano)

10h25 Stability and lytic activity assessment in milk of bacteriophages targeting *Escherichia coli* causing bovine mastitis – Jacob Diderich (University of Liège)

10h45 Coffee pause and poster session

11h15 Second session chaired by Prof. Dr. Damien Thiry – University of Liège

11h20 Exploring determinants of antibiotic prescription behavior among Belgian veterinarians: insights and implications for responsible antibiotic use – Zoë De Mol (Ghent University)

11h40 Udder Health Management and Antibiotic Reduction in Dairy Farming: Introducing Novel Monitoring Tools – Pauline Delhez (RumeXpert)

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12h15 Lunch and poster session

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How to monitor AMR in diseased animals ? The example of the French Resapath network

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JYM is Doctor in Veterinary Medicine, PhD in public health, post-graduate on AMR in human medicine, molecular microbiologist and research director at Anses. He is the Scientific Director of the AMR topic at Anses and Head Unit at Anses Lyon. His research interests focus on molecular genomics and epidemiology of AMR in a One Health perspective and he is an active participant/leader in European/transnational research projects. He is an active contributor to the Resapath network monitoring AMR in clinical animals in France and provides senior level guidance, expertise and support to the implementation of the One Health National Action Plan on AMR and AMU in France, notably in the Agri-Food sector. JYM is a member of several expert groups on policies and strategies on AMR and AMU at national and international level, in particular as former Vice-Chair, and then Chair, of the Scientific Advisory Board of the JPIAMR (2017-2021). JYM is also Head of the FAO Reference Centre for AMR attributed to Anses in 2020.

Pulmonary Aspergillosis in Humboldt Penguins—Susceptibility patterns and molecular epidemiology of clinical and environmental *Aspergillus fumigatus* isolates from a Belgian zoo, 2017–2022

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Introduction

Avian aspergillosis causes a heavy disease burden on birds in captivity, such as Humboldt penguins. In recent years, the colony of a Belgian zoo has experienced high mortality rates with up to 86 % caused by avian aspergillosis. This study was set up to investigate the azole resistance in clinical and environmental *A. fumigatus* strains from the penguin enclosure and whether persistent isolates were present in the penguin habitat.

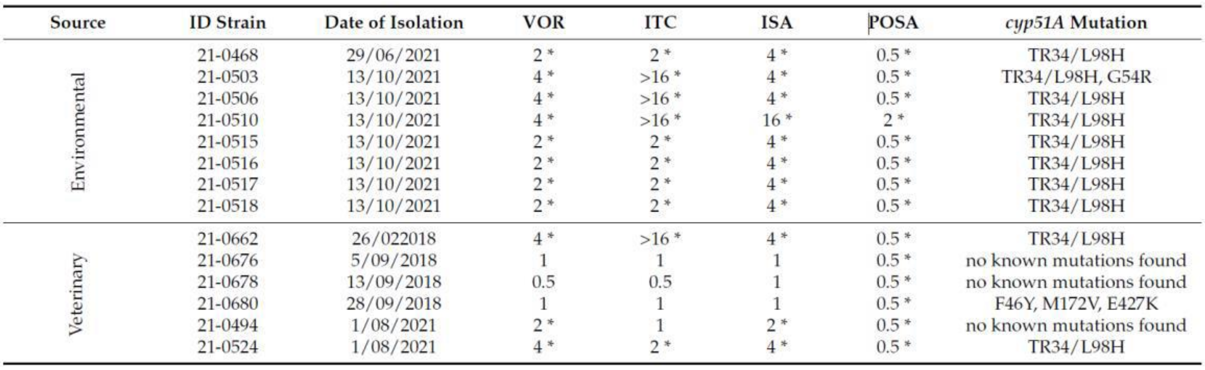
Materials and methods

A total of 35 clinical strains collected from 2018 to 2022 were included in the study. From April 2021 until January 2022, four environmental samplings in the penguin habitat have been performed. A combination of sand, water, nest swabs and air were analyzed for the presence of (azole resistant) *A. fumigatus*. The phenotypical resistance pattern of all isolates was determined using the EUCAST method. Microsatellite genotyping was performed on a selection of 45 *A. fumigatus* isolates.

Results and conclusion

A total of 68 environmental *A. fumigatus* colonies were isolated on MC+T from air samples. No *A. fumigatus* colonies could be detected from the nest swabs, the water samples or nor from the sand. All isolates (n = 97) have been subjected to EUCAST microbroth dilution determination. A total of six clinical (17.14%) and eight environmental (11.76%) *A. fumigatus* isolates displayed resistance against at least one medical azole (Table 1). Cyp51a sequencing of all resistant strains was performed and revealed the TR34/L98H mutation in some isolates, originating from both clinical and environmental strains. The same genotype was found in four environmental strains (21-0515, 21-0516, 21-0517 and 21-0518), and were closely related to two other genotypes: a first one shared by two environmental strains (21-0503 and 21-0510), and a second one corresponding to an isolate (21-0524) originating from a Humboldt penguin. Genotypes within this cluster differed by only one marker (STRAf2A). All seven isolates in this cluster were resistant against at least one medical azole. This study showed high resistance rates in both the clinical isolates (17.14%) and in environmental air samples (11.76%). Genotyping revealed infection by multiple *A. fumigatus* strains in the same penguin individual, as well as a clustering between environmental and veterinary isolates. This study described the first detection of the TR34/L98H mutation in the *Cyp51A* gene in veterinary isolates from Humboldt penguins.

Table 1 – Antifungal susceptibility testing results of the isolates displaying antifungal resistance against at least one medical azole, and associated cyp51A mutation



Source	ID Strain	Date of Isolation	VOR	ITC	ISA	POSA	*cyp51A* Mutation
Environmental	21-0468	29/06/2021	2 *	2 *	4 *	0.5 *	TR34/L98H
	21-0503	13/10/2021	4 *	>16 *	4 *	0.5 *	TR34/L98H, G54R
	21-0506	13/10/2021	4 *	>16 *	4 *	0.5 *	TR34/L98H
	21-0510	13/10/2021	4 *	>16 *	16 *	2 *	TR34/L98H
	21-0515	13/10/2021	2 *	2 *	4 *	0.5 *	TR34/L98H
	21-0516	13/10/2021	2 *	2 *	4 *	0.5 *	TR34/L98H
	21-0517	13/10/2021	2 *	2 *	4 *	0.5 *	TR34/L98H
	21-0518	13/10/2021	2 *	2 *	4 *	0.5 *	TR34/L98H
Veterinary	21-0662	26/022018	4 *	>16 *	4 *	0.5 *	TR34/L98H
	21-0676	5/09/2018	1	1	1	0.5 *	no known mutations found
	21-0678	13/09/2018	0.5	0.5	1	0.5 *	no known mutations found
	21-0680	28/09/2018	1	1	1	0.5 *	F46Y, M172V, E427K
	21-0494	1/08/2021	2 *	1	2 *	0.5 *	no known mutations found
	21-0524	1/08/2021	4 *	2 *	4 *	0.5 *	TR34/L98H

*phenotypical resistance according to the EUCAST clinical breakpoints (v10.0)

Stability and lytic activity assessment in milk of bacteriophages targeting *Escherichia coli* causing bovine mastitis

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Introduction

Bovine mastitis is a major production disease in dairy cattle and complementary treatments to antimicrobials are urgently needed. Intramammary phage therapy is a promising approach but characterizing isolated phages in milk is a crucial initial step. This study aimed to compare the stability and lytic activity of 10 phages targeting *E. coli* in milk and assessing their stability at different temperatures and pH.

Materials and methods

Ten bacteriophages isolated from wastewater were spotted on 53 *E. coli* strains isolated from bovine mastitis to evaluate their host range. The stability of the phages was evaluated across different pH (2-12) and T°C (25-60°C). Stability in milk was assessed after 6h of incubation at 37°C. The optimal multiplicity of infection (MOI) was determined for each phages and used in subsequent lytic activity assays. These assays involved inoculating milk with *E. coli* and phages at the determined MOI and tracking bacterial titers at different timepoints in raw, heat-treated and UHT milk. Illumina sequencing of the phages was performed.

Results and conclusion A narrow host spectrum was observed for the phages and stability was maintained at pH ranging from 4 to 10 and temperatures ranging from 25 to 45°C. At 60°C, only 5/10 phages persisted but with a significant degradation. Stability analysis in milk showed that all phages remained stable in raw and heat-treated milk. Lytic activity assays demonstrated a bacterial decrease with all phages, but for 5/10 phages, bacterial regrowth occurred after 5h of incubation. Regarding the genomic characterization, 2/10 phages presented lysogeny-associated genes.

In conclusion, milk components are not an obstacle for phage therapy to control bovine mastitis. However, bacterial regrowth suggests the presence of resistances that could be bypassed with the use of phage cocktails. Eight phages demonstrated lytic-associated genes, which are required to ensure the treatment safety. Sequencing the bacterial collection will enable us to determine their serotype and associate them with the phage host range, which are linked.

Acknowledgements

This program benefited from a financial support of Wallonia in the frame of a BioWin’s Health Cluster and Wagralim’s Agri-food Innovation Cluster program (Vetphage). The authors acknowledge the Regional Agency of Animal Health and Identification (Ciney, Belgium), the Care-FEPEX (Liège, Belgium) and the “Association intercommunale pour le démergement et l’épuration des communes de la province de Liège” (AIDE, Liège, Belgium).

Exploring determinants of antibiotic prescription behaviour among Belgian veterinarians: insights and implications for responsible antibiotic use

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Introduction

Antimicrobial resistance poses a global threat to public health, with antibiotic usage (AMU) identified as a major driver. Despite successful initiatives to decrease AMU, Belgium still exceeds the European average. The majority of AMU occurs in livestock animals. However, the use of critically important antibiotics is more common in companion animals. Promoting responsible AMU in both livestock and companion animals is needed. Therefore, this study aimed to explore antibiotic prescription determinants among Belgian veterinarians.

Materials and methods

A qualitative exploratory study was conducted involving focus groups with purposefully sampled veterinarians in Belgium. The focus groups were transcribed verbatim, followed by inductive thematic analysis.

Results and conclusion

Nineteen companion animal, seven poultry, five pig, and eleven ruminant veterinarians participated in seven focus groups and one in-depth interview. Thematic analysis uncovered four themes for companion animal veterinarians (the principles guiding the decision-making process, the selection of treatment options, communication within and across practices, and initiatives to promote responsible AMU) and five for livestock veterinarians (the established diagnosis, the available therapeutic options, the veterinarian’s characteristics, the farmer’s and farm’s characteristics, and initiatives to promote responsible AMU) regarding antibiotic prescription behavior.

Participants stated that veterinary medicine has evolved, with a shift from systematic AMU towards prevention, alternatives for antibiotics, and the use of diagnostic tools. However, financial and time constraints were highlighted as barriers. Participants recognized the significance of client-veterinarian communication. They noted a traditional veterinarian-centered approach but emphasized the need for effective communication and mutual trust, with consideration of the client’s perspective. Although more time-consuming, this relationship-centered approach was seen as beneficial for promoting responsible AMU in the long term. Targeted education and campaigns were deemed necessary due to perceived variability in awareness among veterinarians, farmers, and pet-owners. Legislation has been pivotal in shaping decision-making processes among participants in the livestock sector, with regulations on AMU in place since 2016. This has prompted a change in mindset among livestock veterinarians regarding AMU. However, participants also highlighted the necessity for a similar shift in mindset among veterinarians concerning upcoming legislation on AMU in companion animals, scheduled for implementation from September 2024. In livestock, periodic AMU reports were deemed valuable, but user-friendly data collection, balancing animal welfare with decreasing AMU, and financial support were stressed. While familiar with guidelines, veterinarians stressed that their decision-making relies more on their expertise, valuing therapeutic freedom. In companion animals, participants advocated for guidelines across and within veterinary practices promoting a uniform approach and facilitating a change in mindset towards responsible AMU.

The identified themes provide a framework for understanding the dynamics of antimicrobial usage and prescription behaviour in veterinary medicine, revealing both barriers and facilitators for responsible AMU. These insights suggest potential areas for targeted interventions.

Udder Health Management and Antibiotic Reduction in Dairy Farming: Introducing Novel Monitoring Tools

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Introduction

Maintaining optimal udder health in dairy cows is of utmost importance, as it significantly influences the overall welfare of the herd and the economic viability of dairy farms by sustaining high milk production and quality standards. Effective udder health management not only promotes the longevity and productivity of individual cows but also contributes to broader efforts to reduce antibiotic usage in dairy farming. Selective dry cow therapy (SDCT) has emerged as a promising approach, focusing on administering intramammary antibiotics during the dry period solely to cows at high risk of mastitis. Complementary strategies involve regular udder health monitoring for the timely detection and appropriate management of mastitis and other udder infections. In addition to hygiene and sanitation, nutrition and management practices also play role as it supports the cow's immune system. In this context, the development of robust monitoring tools becomes imperative.

Novel Monitoring Tools and Their Implications

To address this need, we have developed three monitoring tools as additional features of SALVE - an online Veterinary Alert System for Livestock developed by RumeXperts. SALVE consolidates and offers intelligent and automated analysis of diverse data concerning cow herds, tank milk, individual milk composition, and weather. These new tools are designed to support the adoption and evaluation of SDCT practices on farms, facilitate the monitoring of udder health, help identify the origin of increased udder infections, and track population trends. This way dairy farmers can optimize antibiotic usage, thereby reducing antimicrobial resistance while promoting animal welfare and safeguarding public health.

The three innovative tools designed to promote udder health are:

- SDCT Monitoring Tool: This tool features individual animal tags integrated into the milk recording module of SALVE, allowing quick filtering and identification of cows eligible for SDCT. The tag is based on somatic cell count (SCC), and statistics from 33,800 milk records and 23 herds revealed that 65% of cows exceeding 350 days in milk were eligible for SDCT.
- Cure and Infection Rate Monitoring Tool: this tool consists of four indicators based on SCC measured during milk recording and calculated at the herd level: (1) cure rate and (2) new infection rate at the last milk recording as well as (3) cure rate and (4) new infection rate at dry-off. These indicators provide valuable insights into overall udder health and mastitis incidence.
- Visualizations and Alerts for Covariation between SCC and Urea or De Novo Fatty Acids: this tool helps identify nutritional causes of mastitis by analyzing covariation between SCC and urea or *de novo* fatty acids in tank milk over time. In an analysis of 196 farms over a 2-year period, half of the farms showed positive covariations between SCC and urea for more than 35% of the time, while half also showed negative covariations for at least 33% of the time. Similarly, half of the farms showed positive covariations between SCC and *de novo* fatty acids for more than 30% of the time, while half also showed negative covariations for more than 39% of the time. There were little difference between seasons.

By leveraging these integrated monitoring tools, stakeholders can optimize udder health management, aligning with antimicrobial stewardship goals and improving bovine health outcomes.

In vivo testing of the NeoGiANT extract-based formulations in poultry

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Introduction

Given the emerging challenge of antibiotic resistance in both human and animal populations, and the diminishing effectiveness of conventional antimicrobial treatments, there's a need for farmers to minimize the use of antibiotics in livestock. Furthermore, there's a critical demand for alternative approaches to treat infection diseases. To address these challenges and to assist livestock farmers in maintaining healthier animals with reduced antibiotic reliance, NeoGiANT proposes an innovative solution leveraging the potent natural antimicrobial and antioxidant properties found in grape marc extracts, rich in a variety of phytochemicals. The NeoGiANT consortium formulated poultry feeds supplemented with varying concentrations of this extract and evaluated their efficacy based on health, performance, and microbiological parameters. This approach holds promise for combatting antimicrobial resistance and supporting sustainable livestock farming practices.

Materials and methods

After developing and testing the solid extract (e.g. for stability), premixes were produced and mixed into the feed. The concentrations of NeoGiANT extract are determined based on the levels of polyphenols (PF), which serve as the active compounds with antimicrobial and antioxidant properties. The polyphenolic content of the feed was analysed to confirm the presence of polyphenols in the feed. In a first dose finding study, efficacy and tolerance were evaluated. Male birds (Cobb 500, n=400) were equally divided amongst four treatment groups (200 mg/kg PF; 750 mg/kg PF; 1500 mg/kg PF; control). In a second dose finding study, male birds (Ross 300, n=400) were equally divided amongst five treatment groups (750 mg/kg PF; 1500 mg/kg PF; control; 1500 mg/kg PF + oral coccidiosis and no coccidiostats in feed; control + oral coccidiosis and no coccidiostats in feed). Productive performance (growth, feed intake, feed-to-gain ratio) were measured throughout the 35 day feeding period. After slaughter, samples were taken for apparent praecaecal and total-tract digestibility. Breast muscle and liver samples were taken for analysis of antioxidant capacity (TBARS). Jejunum and caecum tissue were taken for gene expression (immune response and barrier function) and histology. Caecum and crop content were taken to determine polyphenolic content. Caecum content and faeces were taken for microbiological analysis; i.e. culture-based analysis and metagenomic analysis.

Results and conclusion

Polyphenols from the NeoGiANT extract were (proportionally) present in the supplemented feed and could not be detected in the control feed. No polyphenols from the Neogiant extract could be detected in the crop from the control group, whereas the polyphenols were (proportionally) present in the treatment groups. None of the polyphenols previously detected in feed and crop have been detected in the caecum samples. In both experiments, the use of the four different concentrations of the solid NeoGiANT extract did not show visible effects on broiler performance during the 35 day experiment. The broilers receiving oral coccidia, weighed significantly lower then the birds in the other 3 groups, but there was no significant difference between the supplemented and non-supplemented group. No effect could be shown on apparent ileal digestibility of crude protein and ashes. Expression of genes related to immune response and barrier function and epithelial morphology in jejunum tissue were not significantly different. Goblet cells count in jejunum villi and crypt was higher in broilers receiving 750 and 1500 ppm PF. Thiobarbituric Acid Reactive Substances (TBARS) levels were siginificantly lower in the 1500 ppm group. For total aerobic bacteria, *Enterobacteriaceae* and *E. coli*, there were no significant differences between the treatment groups for neither the faecal nor the caecal samples. In both the caecal and faecal samples, the amount of *Enterobacteriaceae* tended to be the lowest (p>0.05) in the highest dose group (1500 ppm). Further research will be conducted to determine the most effective dosage and potential positive effects on the immunology status of the animals. In addition, the promising results on improved antioxidant status and gut health will be further investigated.

The authors acknowledge i-Grape Laboratory for providing the grape marc extract utilized in this study.
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 101036768.

Abstracts for poster presentations

In vivo testing of the NeoGiANT extract-based formulations in calves

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Introduction

In light of the escalating challenge of antimicrobial resistance in both human and animal populations, along with the diminishing efficacy of conventional antimicrobial treatments, there is a pressing need for farmers to minimize antibiotic usage in livestock production. Additionally, there is a critical demand for alternative approaches to tackle infectious diseases in cattle. To address these challenges and support livestock farmers in maintaining healthier animals with reduced reliance on antimicrobials drugs, NeoGiANT is testing an innovative solution, harnessing the potent antimicrobial and antioxidant properties of grape marc extracts. The NeoGiANT consortium has developed calf feeds supplemented with white grape marc extract and assessed their palatability and safety prior to a larger feeding study to asses health, performance, and microbiological parameters. This approach holds promise for addressing antimicrobial resistance concerns and fostering more sustainable practices in calf and other liverstock production systems.

Materials and methods

A preliminary trial was performed to ensure safety and palatability of the extract. The concentrations of NeoGiANT extract are determined based on the levels of polyphenols (PF), which serve as the active compounds with antimicrobial and antioxidant properties. After an acclimatisation period of 7 days, 9 Holstein-Frisian calves of 70 ± 15 days were randomly assigned to two treatment groups: one receiving 1500 ppm of the extract (n=6) and the other serving as the control group (n=3). Concentrates were soaked in liquid extract to achieve a concentration of 1500 ppm PF. Each calf received approximately 1.0 kg of the concentrate, twice a day. Throughout the 28-day supplementation period, all animals were monitored twice daily to detect any adverse reactions to the supplement or the presence of other infections. Animals were weighed weekly, temperature was measured daily and diarrhoea, appetite, behaviour, body condition and demeanour was scored daily. Serum and faeces were collected weekly for respective immunological analysis and microbiological analysis. On day 28, animals were sacrifized and rumenal content, small intestinal content, caecal content and faeces were collected for detection of polyphenols and its metabolites and microbiological analysis; i.e. culture-based analysis and metagenomic analysis. Liver and kidneys were weighed and assesed for abnormalities by a veterinary pathologist.

Results and conclusion

The feed supplemented with the liquid NeoGiANT extract seemed palatable and safe, as no abnormal behavior or clinical signs could be detected. No difference in liver or kidney weight was observed between supplemented or control groups. In this small preliminary trial, no differences in body weight gain between the control and supplemented group (1500 ppm PF) were detected. Microbiological analysis revealed that the applied polyphenolic extract did not cause a significant change or shift in the faecal microbiota composition of healthy cows. Further samples including those related to immunology are still under investigation. In addition, QTOF mass spectrometry will be employed to analyze polyphenols and their metabolites in various segments of the calf's gastrointestinal system. This approach aims to enhance our understanding of the bioavailability and degradation of polyphenols within the digestive tract. These results provide a foundation for further research into potential positive effects of polyphenols on performance, immunology and health status. Furthermore, to explore properties of the extract in pathogen carriage reduction, calves will undergo a challenge with *E. coli* O157, and both, health status and bacterial fecal shedding will be closely monitored.

The authors acknowledge i-Grape Laboratory for providing the grape marc extract utilized in this study.
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 101036768.

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 NIDHI 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.

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

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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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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}, $bla_{OXA\text{-}48}$, 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}, $bla_{OXA\text{-}48}$, 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 $bla_{NDM\text{-}5}$ (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 of 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.

Acknowledgements

We gratefully acknowledge the ScorWelCow project led by Elevéo (awé groupe) and supported by the Walloon region for providing the data.

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 meningitis, polyarthritis, endocarditis, pneumonia, septicaemia, and polyserositis. Additionally, some strains of *S. suis* can be pathogenic to other species such 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 septicaemia 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 septicaemia (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