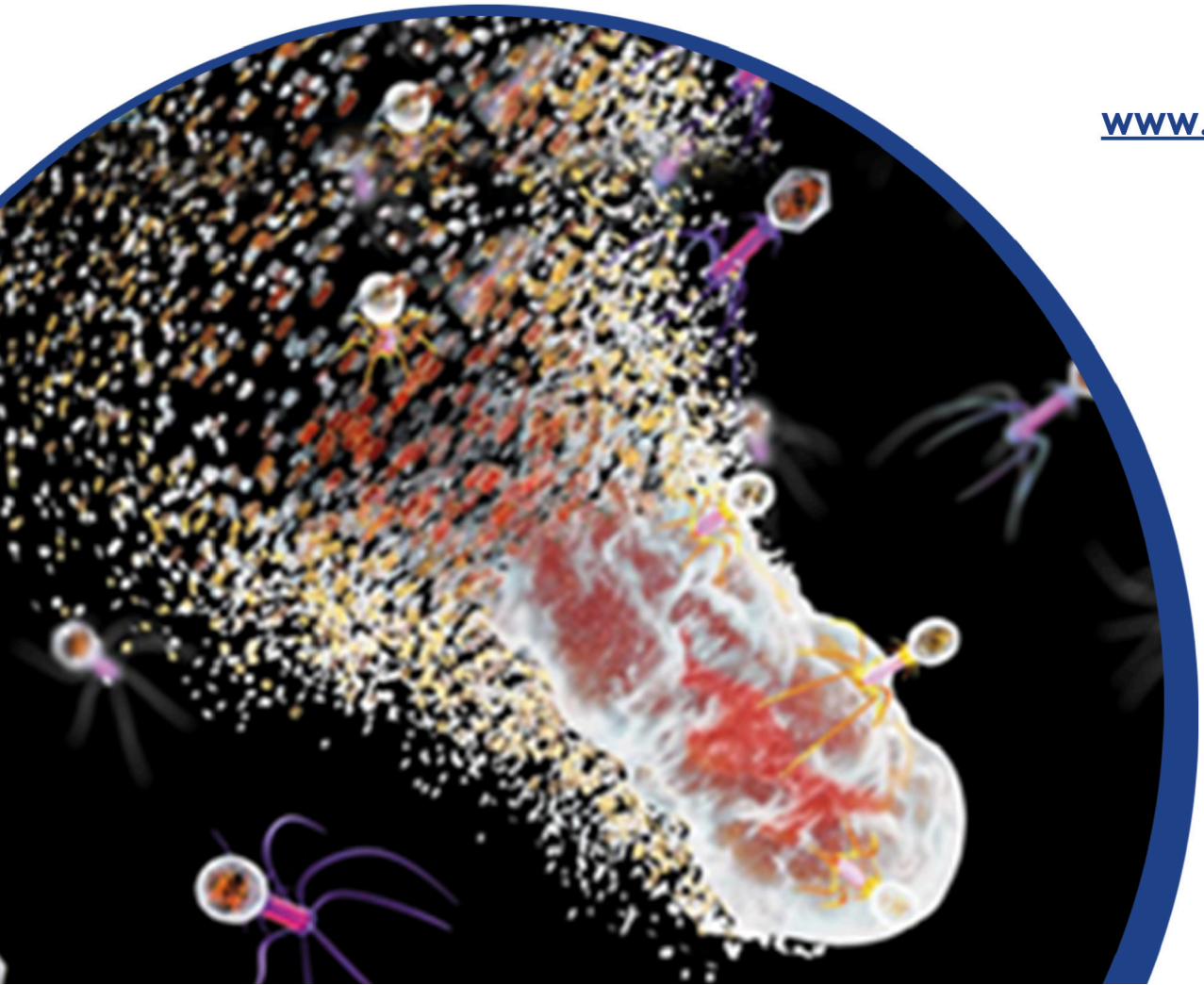
SCIENTIFIC Conference e-Book

International Alliance for Biological Standardization

Avoiding Antimicrobial Resistance: Veterinary Use of Phages for Prevention, Therapy and Control of Bacterial Infections

November 19 & 20, 2024

A virtual meeting



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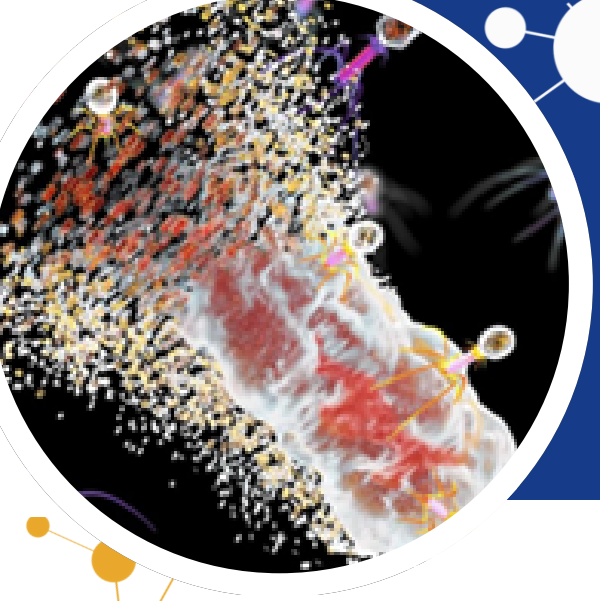
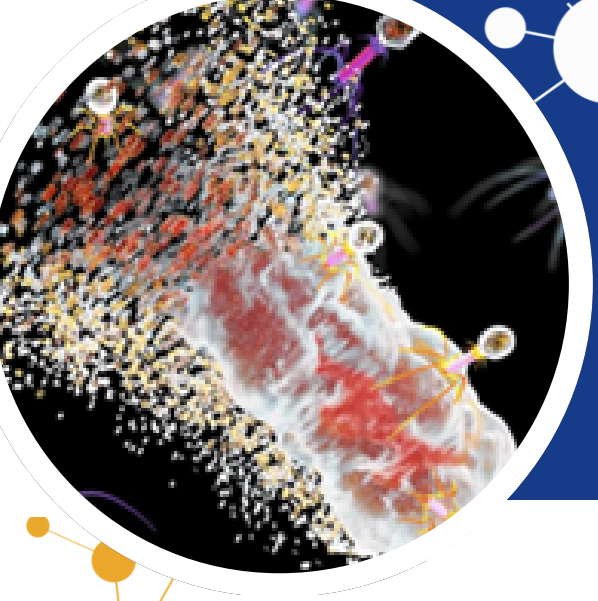


Table of Contents



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Back to Table of Contents

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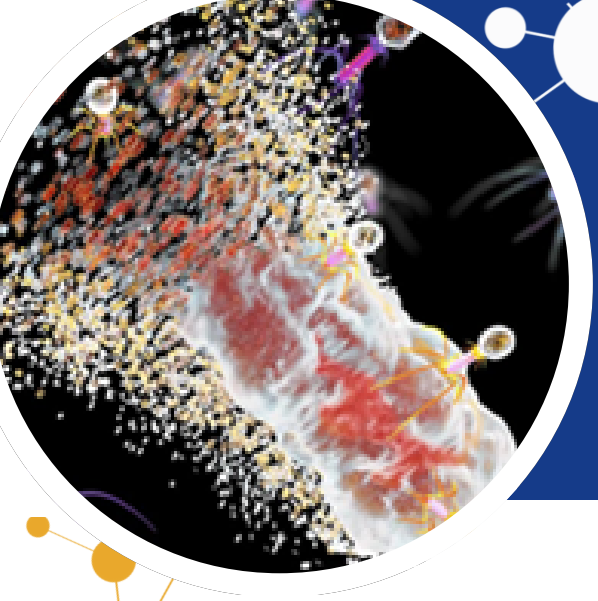


About the Conference

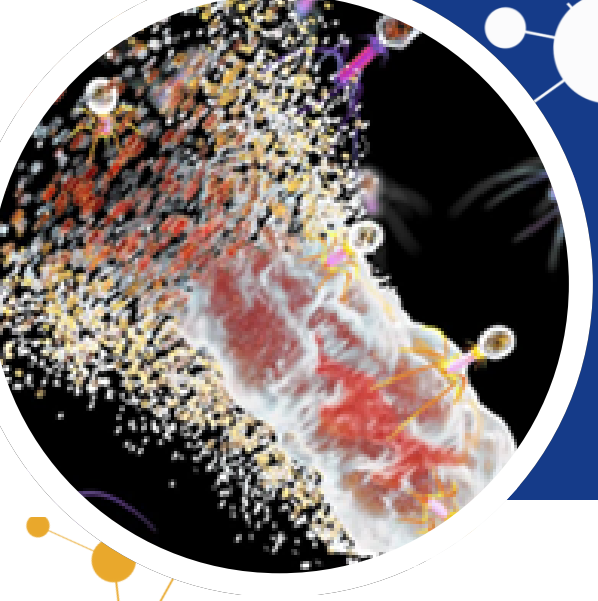
Back to Table of Contents

The Veterinary Biologicals Committee (VBC) of the International Alliance for Biological Standardization (IABS; for description see below) will hold a scientific workshop on quality, safety and efficacy of bacteriophages for veterinary use.
Veterinary medicine is confronted by the need to substantially reduce the use of antimicrobials as part of a joint effort by both human and veterinary medicine (One Health) to fight against antimicrobial resistance (AMR). Bacteriophages used alone, or in combination with antimicrobials are a supplementary tool to conventional antibiotics and have been developed for use in several areas, e.g. in aquaculture, bovine mastitis, pig diarrhoea, control of salmonella in pigs, as well as for treatment of conditions in individual animals such as dermatitis in dogs.

Therefore, the workshop will focus on the use of phages to reduce antimicrobials use:

- Therapeutic use of phages in production and companions animals;
- Prophylactic use of phages with the aim to reduce infectious pressure in animal holdings.

This workshop will create an ideal environment for discussion between regulators, academics and companies to facilitate the development of phages as an additional tool to reduce AMR and as a therapeutic tool for individual/herd treatment of resistant bacterial infections.
This meeting of the different stakeholders should promote a better understanding between regulators and companies developing phages: regulators can explain the existing regulatory framework (e.g. EMA guideline and EP chapter) and discuss their interpretation; companies can explain the drawbacks/difficulties in the industrial development of phages and possible solutions. This meeting will conclude with concrete suggestions on how access to market for phage products can be improved.



Scientific and Organizing Committee

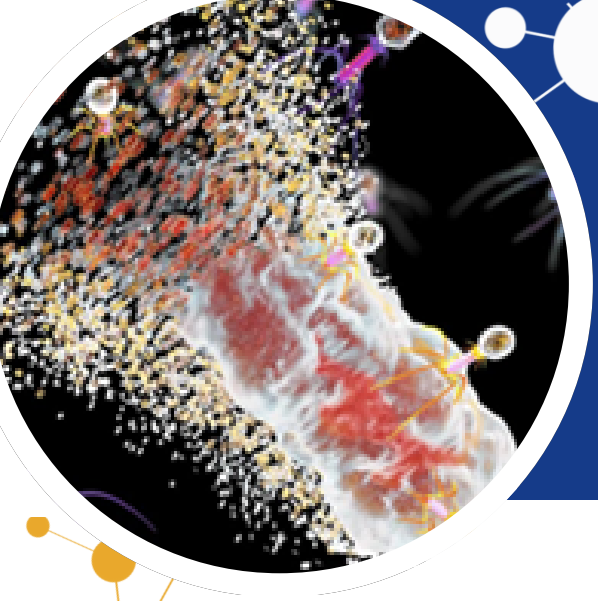
Back to Table of Contents

Scientific Committee

Etienne Thiry - Chair, VBC vice chair, ULiège
Miia Jakava-Viljanen - VBC chair, Finnish Food Authority
Carmen Jungbäck - VBC member, IABS board secretary
Hans P. Kleppen - VBC member
Bryce Lunt - Zoetis
David Mackay - VBC member
Paul Midtlyng - VBC member, Aquamedic AS
Egbert Mundt - EVA Pharma
Dusan Palic - VBC member, U München
Jean-Paul Pirnay - Queen Astrid Military Hospital
Damien Thiry - ULiège
Jeffrey L. Watts - Zoetis

Organizing Committee

Etienne Thiry - Chair, VBC vice chair, ULiège
Miia Jakava-Viljanen - VBC chair, Finnish Food Authority
Carmen Jungbäck - VBC member, IABS board secretary
Madinina Cox - IABS Secretariat
Marlène Louis - IABS Secretariat



Scientific Program

Tuesday, November 19, 2024

Back to Table of Contents

1:30 - 2:00 *Technical and Organizational matter*

2:00 - 2:10 Opening of the meeting - Welcome and introduction to the meeting
Etienne THIRY, ULiège, Belgium

KEYNOTE SESSION

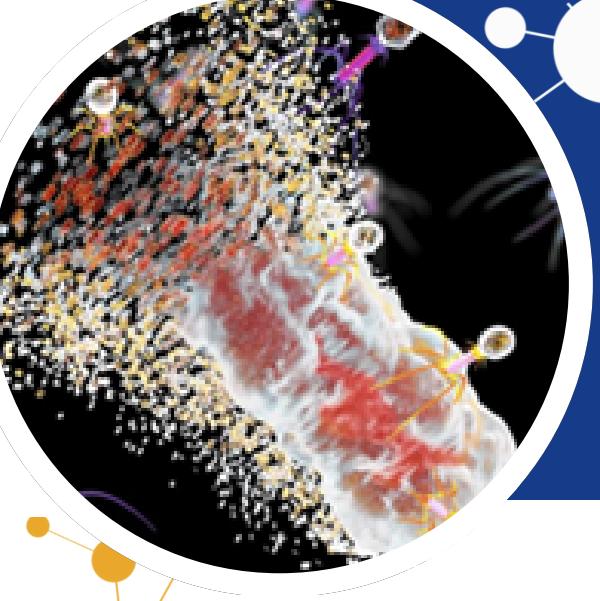
2:10 - 2:50 Keynote lecture: Phages and phage proteins in bacterial infection treatment
Yves BRIERS, Ghent University, Belgium

Session I: Field use of phages to improve animal and human health: from population to individual treatment

Moderators: Paul MIDTLYNG, Dusan PALIC

2:50 - 3:10 Use of phage in aquaculture: are we treating the water or the animals?
Hans KLEPPEN, Norway

3:10 - 3:30 Towards bacteriophage control of Campylobacter in the poultry rearing environment
Ian CONNERTON, University of Nottingham, UK

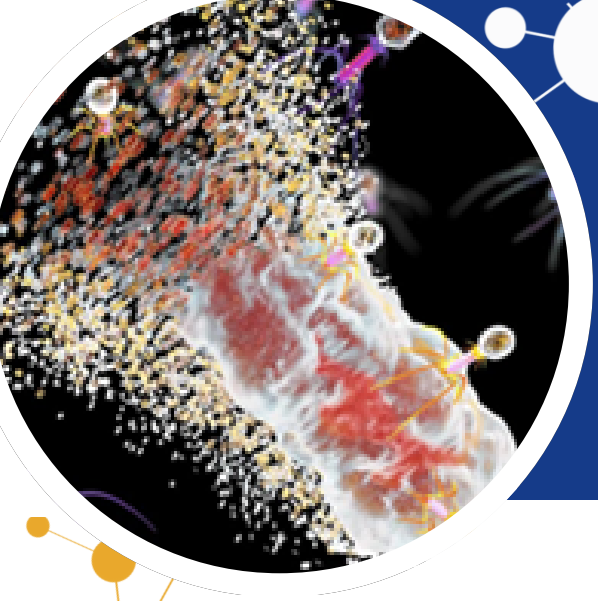


Scientific Program

Tuesday, November 19, 2024

Back to Table of Contents

3:30 - 3:50	Phage therapy in pigs: observations on the control of zoonotic pathogens in pork production **Robert ATTERBURY**, University of Nottingham, UK
3:50 - 4:20	Break
4:20 - 4:40	A bacteriophage product against *Staphylococcus* aureus to treat bovine mastitis **Hansjörg LEHNHERR**, Phage Technology Center, Germany
4:40 - 5:00	Phage therapy in humans, the "individual patient" approach **Sarah DJEBARA**, Queen Astrid Military Hospital, Belgium
5:00 - 5:20	Phage therapy in companion animals: which way for an efficient treatment? **Damien THIRY**, ULiège, Belgium
5:20 - 6:00	Q&A Session 1
6:00	End of Day 1



Scientific Program

Wednesday, November 20, 2024

Back to Table of Contents

1:00 - 1:05

Welcome address
Etienne THIRY, ULiège, Belgium

Session II: Poster Session

Moderators: Etienne THIRY

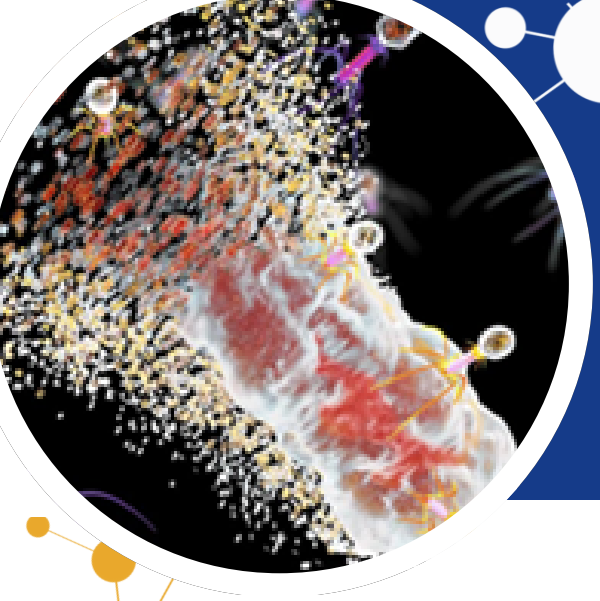
1:05 - 2:00

Exploiting Campylobacter jejuni phage receptor binding protein for rapid and specific bacterial detection.
Su Zar Chi LWIN, Graduate School of Bioresource and Bioenvironmental Sciences, Japan

In vitro efficacy and individual bacteriophage treatment of P. aeruginosa in dogs
Eva Maria KALBHENN, Phage Center Bad Kissingen, Germany

Identification of effective bacteriophages for mastitis prevention in goats
Audrey ADDABLAH, Laval University, Canada

Alphagos Vibrio , a tailored phage cocktail reduces Vibrio parahaemolyticus infection in Indonesian shrimp hatcheries
Agniah, PT Vaksindo Satwa Nusantara, Indonesia



Scientific Program

Wednesday, November 20, 2024

Back to Table of Contents

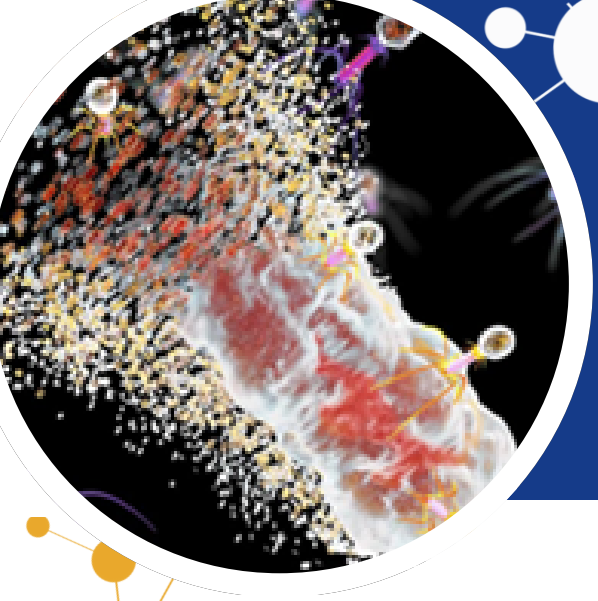
Therapeutic efficacy of bacteriophage cocktail against multidrug resistant Streptococcus agalactiae infection in Oreochromis niloticus
Prenanka RAJAN, Kerala University of Fisheries and Ocean Studies (KUFOS) Ernakulam, India

High performance genome annotation for a safer and faster-developing phage therapy
Guillaume ABRIAT, Rime Bioinformatics SAS, France

Alphagos, a tailored phage cocktail, controls multiple-Salmonella enterica strain colonization in broiler chickens
Ilias THEODOROU, Phagos SAS, France

Alphagos, a tailored phage cocktail, reduces Salmonella enterica colonization in chicken carcasses
Marine FEYEREISE, Phagos SAS, France

K1 Capsule-dependent phage-driven evolution in Escherichia coli leading to phage resistance and biofilm production
Céline ANTOINE, ULiège, Belgium



Scientific Program

Wednesday, November 20, 2024

Back to Table of Contents

Session III: Panel discussion: How to bring phage products for the treatment of animals to market?

Moderators: David MACKAY, Jean-Paul PIRNAY, Hans KLEPPEN

2:00 - 3:30

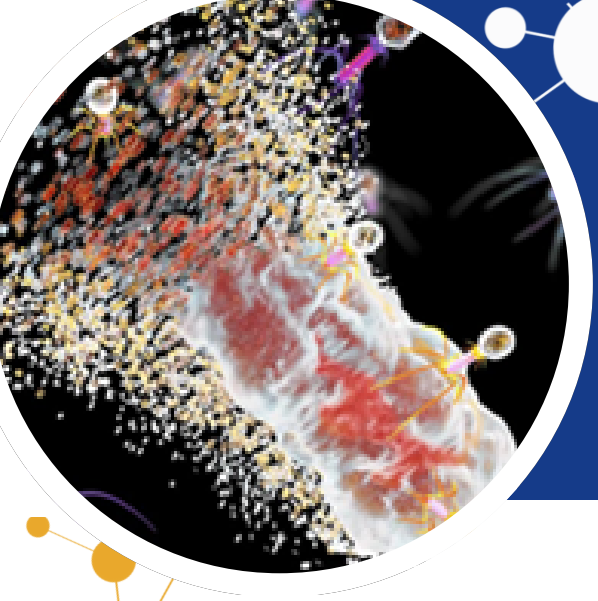
Introduction
David MACKAY

Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy
Rosario BULLIDO & **Susana CASADO**, Spanish Agency of Medicines and Medical Devices (AEMPS), CVMP Novel Therapy WP, Spain

Activities of the European Pharmacopoeia in the field of bacteriophages
Olga KOLAJ-ROBIN, European Directorate for the Quality of Medicines & Healthcare, France

Regulating phages as veterinary medicines in the USA; Eligibility, requirements and challenges
Emily CORNWELL, CVM, USA
Bruce THOMSEN, MRP-APHIS, USA

EFSA's experience in the risk assessment of bacteriophages intended to be used as feed additives
Rosella BROZZI, European Food Safety Authority (EFSA), Italia



Scientific Program

Wednesday, November 20, 2024

Back to Table of Contents

Quality Control of Therapeutic Phages in Belgium
Pieter-Jan CEYSSENS, Sciensano, Belgium

The Importance of CMC for Bringing Animal Health Phage Products to Market
Jeffrey L. WATTS, Zoetis, USA

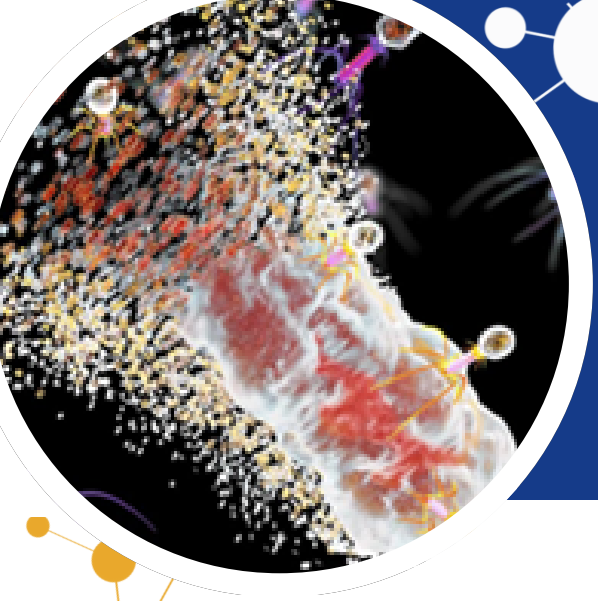
Phage therapy for veterinary applications from the perspective of a manufacturer
Johan QUINTENS, Vésale Bioscience, Belgium

3:30 - 4:00 Break

4:00 - 5:30 Panel Discussion
Moderators: Paul MIDTLYNG, David MACKAY

Examples of topics:
- How to choose the appropriate pathway?
- What are the quality requirements, including manufacture / GMP that apply?
- Are requirements for safety and efficacy sufficiently defined?
- Scientific challenges that the panel would like to draw attention to?

5:30 Closing remarks - End of the meeting



Upcoming IABS Conferences and Workshops

Back to Table of Contents

2024

4th Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animal

NGS Training Workshop
December 3, 2024
4th NGS Conference
December 4 & 5, 2024
Frankfurt, Germany

2025

Advances in Analytical Technologies for Biopharmaceutical Products

Rockville, MD, USA
March 19-21, 2025

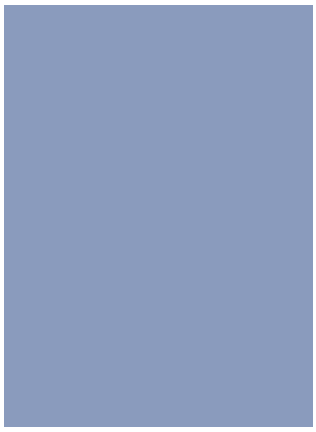
Leveraging Analytical and Bioprocess Platforms for Biological Product Development and Commercialization

Brussels, Belgium
May 14-15, 2025

Cross Learning Experience Human and Animal Vaccine Licensure based on Technology Platforms

Brussels, Belgium

Biosketch



Yves BRIERS

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Yves Briers is an Associate Professor and head of the Laboratory of Applied Biotechnology at the department of Biotechnology of Ghent University (Belgium). The research interests of his group focus on the synthetic biology of modular proteins with a particular focus of phage proteins such as lysins and phage receptor binding proteins. He co-founded the Belgian Society for Viruses of Microbiology in 2022, which he currently chairs. Last year in summer 2023 he founded the spinoff Obulytix that develops phage lysins for human therapy, for which he is currently acting as parttime CSO.

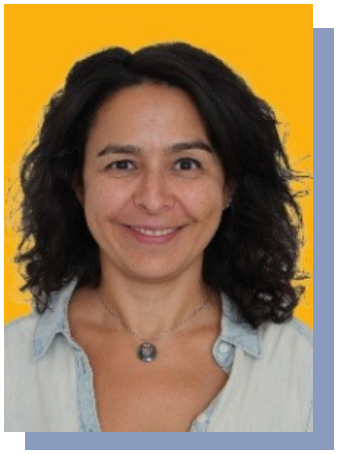
Abstract

Yves Briers

Phages and phage proteins in bacterial infection treatment

Bacteriophages (phages) are viruses that specifically target bacteria. Phage therapy involves using phages to treat bacterial infections. These viruses infect and replicate within bacterial cells, ultimately leading to bacterial death. Phages have been studied extensively for their potential in human and veterinary therapy. Additionally, phage-derived proteins, particularly endolysins and depolymerases, are actively investigated as alternative or supportive antibacterials. Endolysins rapidly degrade the bacterial cell wall, while depolymerases target specific components of the bacterial surface such as capsule or LPS. In this talk, the basics of phages, endolysins and depolymerases will be explained with practical cases on their use for therapy in mastitis and pyoderma.

Biosketch



Rosella Brozzi

Scientific Officer, Feedco Unit,
Department of Risk Assessment
Production (ASSESS), European Food
Safety Authority (EFSA)
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E-mail: rosella.brozzi@efsa.europa.eu

Rosella Brozzi holds a master's degree in food science, technology and legislation from the University of Parma (Italy). Initially she worked in the microbiological laboratory of a dairy multinational company. In 2004 she joined the European Commission. She was responsible for the coordination of the relations with the European Food Safety Authority (EFSA) and for the food additives' legislation. Since 2007 she is working in EFSA as a Scientific Officer in the FEED team of the Feed and Contaminants unit which provides support to the Panel on Additives and Products or Substances used in Animal Feed (FFEDAP). The FEEDAP Panel provides scientific advice to risk managers on the safety and/or efficacy of additives and products or substances used in animal feed. The FEEDAP Panel evaluates their safety and/or efficacy for the target species, the user, the consumer of products of animal origin and the environment. It also looks at the efficacy of biological and chemical products/substances intended for deliberate use in animal feed. One of Rosella's main tasks is the coordination of the activities of the Working Group on Microbiology. This committee is responsible for the preparatory work regarding the evaluation of microbial-based products, including bacteriophages, subject of applications and of the risk assessment by the FEEDAP Panel.

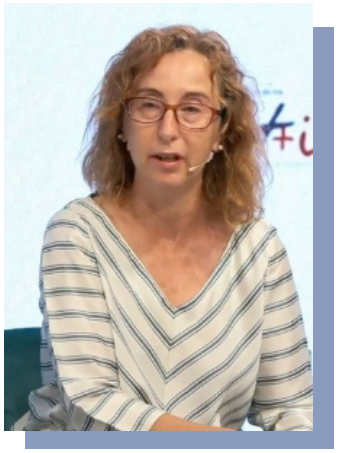
Abstract

Rosella Brozzi

EFSA's experience in the risk assessment of bacteriophages intended to be used as feed additives'

Feed additives are products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin, or to improve the animals' performance and health. Feed additives may not be put on the European Union (EU) market unless an authorisation has been granted following a scientific evaluation demonstrating that the additive has no harmful effects, on human and animal health and on the environment. The European Food Safety Authority (EFSA) is responsible for carrying out this evaluation to support EU risk managers in their decision-making process. The process entails a request for authorisation from business operators wishing to place the product in the EU market which should be accompanied by a technical dossier containing the data underpinning the scientific evaluation and subsequent authorisation. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) provides scientific advice to risk managers on the safety and/or efficacy of additives and products or substances used in animal feed. The FEEDAP Panel evaluates their safety and/or efficacy for the target species, the user, the consumer of products of animal origin and the environment. It also looks at the efficacy of biological and chemical products/substances intended for deliberate use in animal feed. In 2021 the FEEDAP Panel adopted its first opinion on a product composed by bacteriophages and intended for use as a feed additive to reduce the Salmonella contamination in poultry and the environment. In that opinion, the safety of the product could be established, but not its efficacy. A second inconclusive opinion on the efficacy of the product was adopted in 2023. The presentation will address the challenges encountered when assessing the data concerning this phage preparation.

Biosketch



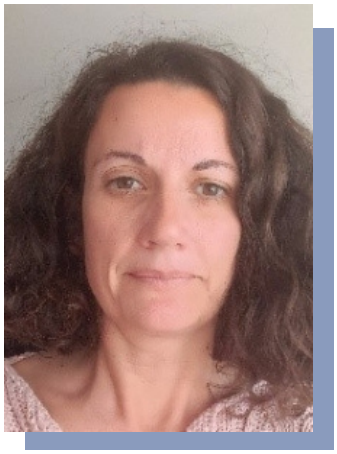
Rosario Bullido, DMV (AEMPS). PhD.

Head of Biologicals Veterinary Medicines
28022 Madrid, Spain
Spanish Agency of Medicines and Medical Devices (AEMPS)
Tel: +34 91 822 54 19
E-mail: rbullido@aemps.es

HEAD OF of BIOLOGICAL IMMUNOLOGICALS Veterinary medicines, including nove therapies in the Spanish Medicines and medical devices Agency (AEMPS). I have been working in the Veterinary Department (DMV) of the AEMPS since 2001. As part of this work I am member of the Novel Therapies Working Party (NTWP) and of the Immunological Working Party (IWP) (EMA) and also expert of the 15V group of veterinary vaccines (EDQM), and member of the Standing Cometee on prequalification of Vaccines against FAST (FMD and similar transboundary animal) diseases.
I have also participate in other groups and in the development and/or revisión of veterinary medicines legislation and guidelines, as in expert group as in Autogenous vaccines (CMDv), Regulation 6/2019 (Annex II), ATA (Alternatives to antimicrobials), Monoclonal antibodies (ADVENT, Novel therapy), etc.

PhD in veterinary immunology (Complutense University of Madrid- CISA-INIA Valdeolmos. Madrid) in porcine immunology. Post-doc fellowship in fish immunology (CISA-INIA) and in human influenza virus molecular biology - Instituto Carlos III. Madird.

Biosketch



Susana Casado, DMV, PhD, MSc

Head of Unit of Biological Veterinary Medicines
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Susana Casado is Head of Unit of Biological Veterinary Medicines in the Veterinary Department of the Spanish Agency of Medicines and Medical Devices (AEMPS).

She is also member of the EMA (European Medicines Agency) working parties dedicated to novel therapies (NTWP, acting as Vice-Chair) and scientific advices (SAWP-V).

She has been hired by AEMPS since 2018 where she assesses marketing authorizations, variations, clinical trials and other regulatory procedures related to biologicals and novel therapies veterinary medicines. She also participates in national and European scientific advices, and in the development and revision of legislation and guidelines applicable of these kind of products.

Susana is Veterinary by training and she also obtained a PhD degree in Veterinary, as well as a Master Degree in Research and Development of Medicines (ESAME).

Before joining the AEMPS, she worked around 20 years in Spanish Research centers (Faculty of Veterinary, Research Institute Carlos III and INIA), and in pharmaceutical companies, in jobs related to regulatory affairs and quality system management for companies focused on in vitro medical devices, immunotherapies and advanced therapies (human/veterinary).

Abstract

Rosario Bullido - Susana Casado

Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy

Currently, bacteriophages are reappearing in the therapeutic arsenal as a potential alternative to antibiotic therapy (or to complement the latter) as a salvage therapy in therapeutic dead end, due to increasing antibiotic resistance.

In the veterinary field, these products can be classified as medicines and require a marketing authorization before commercialisation, as stated in Regulation (EU) 2019/6 of the European Parliament and of the Council of 11 December 2018 on veterinary medicinal products and repealing Directive 2001/82/EC.

Bacteriophages as therapies present certain peculiarities. Their action is linked to their lytic activity, generally restricted to specific bacterial strains. Additionally, the interaction bacteriophage-host bacteria is a dynamic process and host bacteria might develop resistance against bacteriophages with some frequency.

Consequently, VMP based on bacteriophages are expected to require frequent changes in composition for the bacteriophage strain(s) to maintain efficacy/circumvent resistance development in relation to the intended indication.

In order to avoid regulatory constraints due to the variable composition of bacteriophages as medicinal products, the EMA drafted the Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy.

The applicable regulatory/technical and scientific requirements for the marketing authorization of this type of medicines are described there.

The guideline establishes the basis for the authorization of phage products with flexible qualitative and quantitative composition as veterinary medicines.

Abstract

Rosario Bullido - Susana Casado

Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy

In general, the requirements stated in Annex II of Regulation (EU) 2019/6 should be followed. Phage therapy products are classified as novel therapies (NT) and this allows the application of risk-based principles for determining the requirements for the marketing authorization.

The allowed adaptation implies a deep scientific knowledge of the product, the application of risk based approaches, and appropriate design of quality risk management system, and pharmaceutical quality systems.

In the guideline, the concepts of parental bacteriophage products and representative preparations are explained. Both concepts allow the authorisation of medicines with variable composition on bacteriophages.

The guideline would benefit both industry and regulators due to provision of a relevant guidance on data requirements for bacteriophages medicinal products. It will facilitate and speed up the development and authorisation of veterinary medicinal products specifically designed for phage therapy, hence contributing to increase the availability of the veterinary medicinal products tackling with antibiotic resistance.

The development of the guideline involved experts of the OEG (operational expert group) on bacteriophages, the NTWP (Novel therapy Working Party), and the CVMP (Committee for Veterinary Medicinal Products). We would like to thank all the experts collaborating in this work.

Biosketch

Pieter-Jan Ceyssens

Unit Head 'Antibiotics and Resistance
Sciensano
Rue Juliette Wytsman 14, 1050 Ixelles,
Belgique

I obtained a master in Bioscience Engineering, graduated cum laude in 2004 and completed my PhD in Bioscience Engineering at the department of Biosystems (University of Leuven) in 2009. This research was funded by an IWT scholarship and focused on phylogenetics of phages infecting Pseudomonas aeruginosa. Afterwards, and funded by PDM/FWO postdoctoral fellowship (2010-2013), I applied multi-omics approaches for phage-host interaction studies. During his postdoc, I spend research time at the MFPL Laboratories at the University of Vienna (Prof. Udo Bläsi) on transcriptomics, and at the University of Pittsburg (Prof. Graham Hatfull) on phage recombineering.

Since 2014, I am affiliated with Sciensano and was appointed head the Unit 'Antibiotics & Resistance' in 2015. This unit is embedded within the division of Human Bacterial Diseases, which groups National Reference Centers (NRCs) of Salmonella, Shigella, Listeria, Yersinia, Neisseria and Mycobacterial spp. Over the years, I am more and more involved in policy related to public health, antimicrobial resistance and prevention. I am One Health focal point for Antimicrobial Resistance at Sciensano (2018-2023), elected member of the board of directors of the Belgian Society of Microbiology (2021-2027), the Belgian Society of Viruses of Microbes (2022-2026), member of the Belgian Pharmacopeia submission Biology (2021-2026) and chair of the EU working group on the establishment of a General Chapter on phage therapeutics in the European Pharmacopeia.

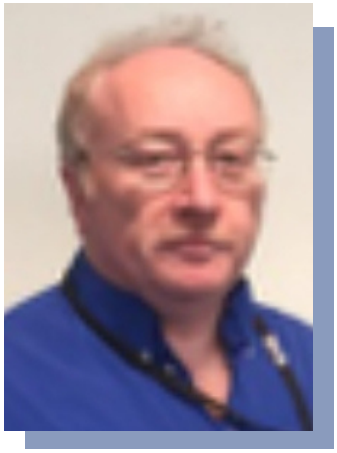
Abstract

Pieter-Jan Ceyssens

Quality Control of Therapeutic Phages in Belgium

The greatest hurdle to the introduction of phage therapy in Western medicine remains the lack of an appropriate legal and regulatory framework. Since 2018, Belgium is implementing a pragmatic phage therapy framework that centers on the unlicensed ad hoc tailor-made phage medicines in magistral preparations. Central to this approach is a two-step quality control of phage active pharmaceutical ingredients (APIs) effectuated by Sciensano. This API QC consists of the construction of a genetic passport which contains information of phages lifestyle, genome size and content, and its capacity for horizontal gene transfer. Secondly, each production lot is checked for identity, potency, microbial contamination, endotoxin levels and pH, using either official Pharmacopeia or in-house protocol. I will briefly discuss the QC pipeline, and relevant aspects in the dedicated Ph. Eur working group.

Biosketch



Ian Connerton

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Ian Connerton is Professor of Food Safety at the University of Nottingham, UK. After undergraduate studies in Biochemistry, he was awarded a PhD in Chemistry from the University of Warwick in 1985. After post-doctoral research at the University of Cambridge in Genetics, he joined the University of Reading in 1987 to teach Microbiology and began his interest in the foodborne pathogen Campylobacter. He joined the Institute of Food Research in 1991 to become Deputy Head of Food Macromolecular Science with specific interests in the structure and function of plant cell wall degrading enzymes. He was appointed as the first Northern Foods Chair of Food Safety at the University of Nottingham in 1998, where he developed his research interests in bacteriophages for the control Campylobacter ssp. from zoonotic sources.

Abstract

Ian Connerton

Towards bacteriophage control of Campylobacter in the poultry rearing environment

BACKGROUND

Campylobacter species are the most common cause of human bacterial gastroenteritis worldwide. Poultry are a reservoir for the pathogen and poultry meat a major source of foodborne Campylobacter. Birds reared for meat and a target for the application of bacteriophages to control Campylobacter at source. Campylobacter bacteriophages are frequently present in poultry production units and can be recovered from retail poultry meat, and are therefore regularly encountered by birds in production facilities and the consumer without harm.

METHODS

In vivo studies, Ross 308 broiler chickens colonized by C. jejuni formed the basis to test the efficacy of phage therapy against Campylobacter. Chickens were colonized at 6 or 20 days of age by oral gavage, and either phage(s) or a placebo administered before colonization as a prophylactic measure or after as active therapy. Campylobacters and phages were recovered from intestinal contents or organs at necropsy to assess colonization levels. The frequency of resistance to bacteriophages post-intervention was assessed using single colonies recovered from primary isolation plates onto which intestinal contents from all phage-treated and control birds had been inoculated. The impact of phage therapy was assessed on the 16S rRNA PCR amplifiable bacterial communities of the gut using DNAs isolated from ileal and cecal contents.

Abstract

Ian Connerton

Towards bacteriophage control of Campylobacter in the poultry rearing environment

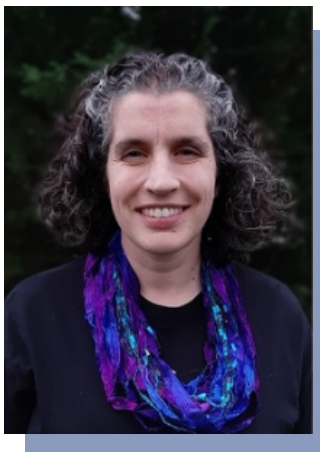
RESULTS

Monophages or phage combinations containing virulent Campylobacter phages significantly reduced Campylobacter counts from the cecal contents of broiler chickens ($p<0.05$). The reductions observed varied with the phage and the phage titres applied but ranged between 1 and 5 log10 CFU/g. Reductions in the colonization levels were evident throughout the rearing periods but were most effective 2 days post-treatment. Effective phage therapy coincided with evidence for phage replication in vivo to establish stable populations. Bacteriophage predation of C. jejuni was not found to affect the resident bacterial microbiota but selectively reduced the abundance of C. jejuni.

CONCLUSIONS

Campylobacter bacteriophages can reduce the intestinal carriage of C. jejuni in broiler chickens without any collateral effects on the gut microbiota.

Biosketch



Emily Cornwell, DVM, PhD, CertAqV

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Dr. Emily Cornwell is a Veterinary Medical Officer with FDA's Center for Veterinary Medicine in the Office of Surveillance and Compliance, Division of Drug Compliance. Prior to joining FDA, Emily was a clinical veterinarian in private practice for 5 years after receiving her DVM and PhD from Cornell University.

Abstract

Emily Cornwell

X

X

Biosketch

Sarah Djebara

Doctor
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Medical doctor, specialized in internal medicine (Free University of Brussels) and in tropical medicine and international health (Tropical medical institute of Antwerpen).
Special interest in auto-immune and rare diseases in internal medicine. 2 Diploma: immuno-hémato and rare diseases in internal medicine (UPEC Paris) and Polyarthritis and systemic diseases (Montpelier University)

Since the end of 2017, I'm part time working at the Queen Military hospital where I helped building a phage therapy coordination center and where I'm the medical doctor responsible of the phage therapy coordination.

Abstract

Sarah Djebara

Phage therapy in Belgium

Bacteriophage therapy (BT), or the therapeutical use of phages, is put forward as an additional tool against difficult-to-treat infections.
The place of phage therapy as an adjuvant of antibiotics needs to be determined with prospective studies like Phage Force(1), STAMP(2) studies and well-documented case studies and series(3) are fundamental to standardize our practices and avoid the publication biases that plague the phage therapy field today.
In Belgium, dedicated and pragmatic phage therapy framework was implemented in 2018, which resulted in the facilitation (by the phage therapy coordination center at the Queen Astrid Military Hospital in Brussels) of 171 patients so far. The first 100 consecutive cases were recently analyzed, showing clinical improvement and eradication of the targeted bacteria for 77.2% and 61.3% of infections, respectively. In our dataset of 100 cases, eradication was 70% less probable when no concomitant antibiotics were used (odds ratio = 0.3; 95% confidence interval = 0.127–0.749). In vivo selection of bacteriophage resistance and in vitro bacteriophage–antibiotic synergy were documented in 43.8% (7/16 patients) and 90% (9/10) of evaluated patients, respectively. We observed a combination of antibiotic re-sensitization and reduced virulence in bacteriophage-resistant bacterial isolates that emerged during BT. Bacteriophage immune neutralization was observed in 38.5% (5/13) of screened patients. Fifteen adverse events were reported, including seven non-serious adverse drug reactions suspected to be linked to BT. While our analysis is limited by the uncontrolled nature of these data, it indicates that BT can be effective in combination with antibiotics and can inform the design of future controlled clinical trials. (4)

Abstract

Sarah Djebara

Phage therapy in Belgium

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Biosketch

Miia Jakava-Viljanen

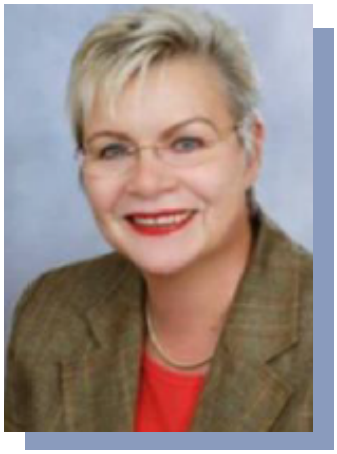
DVM, PhD, Specialist in Animal Disease
Chair, Veterinary Biologicals Committee
Finland

Following positions at the Helsinki University in Finland in teaching microbiology, immunology and epidemiology, and research, as a veterinarian, Dr Miia JakavaViljanen joined the Finnish Food Authority as Head of Section involved in virology, epidemiology and veterinary vaccines, and batch release and testing of vaccines.

She subsequently moved into the policy area, assuming the post of Government Counsellor at the Ministry of Agriculture and Forestry of Finland worked with Animal Health and Welfare legislation, rabies, pet movement, bee health, animals used for scientific purposes (3Rs), EU co-financed programs,funding for research projects and collaboration with Russia. Seconded to the European Commission, she was involved in the implementation of the EU legislation on Animal Health and participated to the work on EU climate change.

She joined the European Medicines Agency in 2014 - 2019 as National Expert where she was responsible to provide consultation and expertise specifically in the area of veterinary biologicals, immunological medicines and emerging therapies, EU legislation and policy as scientific/content lead. Currently she is working at the Finnish Food Authority.
She is an expert of EDQM European Pharmacopoeia Group 15V (vaccines and sera) since 2002. She joined IABS in 2019 and is a member of the executive board and the Chair of the Veterinary Biologicals Committee. She is organizing IABS meetings focusing on the veterinary field.

Biosketch



Carmen Jungbäck, Dr

Board Member Vice-Chair
IABS-EU
USA

Dr Carmen Jungbäck graduated from the Tierärztliche Hochschule, Hannover with a degree in Veterinary Medicine. In 1981, after a few years as an animal surgeon she joined the Paul-Ehrlich-Institut, (Federal Agency for Sera and Vaccines), Langen, Germany, where she was Head of the section Veterinary Virology 1 until retirement in 2016. The section's area of activities comprises vaccine licensing and testing, with special expertise in viral vaccines for poultry. In this context, the practical testing of vaccines during licensing and for official batch release is one of the major responsibilities.

She was also member of a number of advisory boards to the EDQM-OMCL Network, Ph.Eur Group 15V and CVMP-IWP and JEG3R at EMA dealing with IVMPs under various aspects.

At IABS she is member of the board and Chair of the Veterinary Biologicals Committee and Vice-President of IABS - EU. She is organizing IABS meetings focusing on the veterinary field. As member of IABS-EU she is involved in the IMI projects (ZAPI and Vac2Vac).

Biosketch

Hans Petter Kleppen, PhD

Phage scientist
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I´m a molecular biologist who did my PhD degree at NMBU on bacteriophage-host interactions in industrial food fermentation. Got inspired to work with practical application of bacteriophage. Built and lead the ACD Pharma Bacteriophage group for thirteen years, resulting in the successful development of the phage product series Custus™ which has shown very good results and sales in the Norwegian aquaculture industry. Held positions as senior scientist, CSO and VP for research and development at ACD Pharma and Nordly Holding. Currently on garden leave and trying to figure out what my next phage-based adventure will be.

Abstract

Hans Petter Kleppen

Use of phages in aquaculture: Are we treating the water or the fish?

When used right, bacteriophages can be safe and efficacious antibacterial solutions, and important supplements to other strategies, such as hygiene, vaccines and antibiotics.

The bacteriophage product Custus®YRS contains lytic bacteriophages which specifically infect and kill Yersinia ruckeri, the bacterium causing yersiniosis disease in salmon farms. Typically, Yersinia ruckeri infects a subset of a fish population very early in life, establishing a subclinical carrier state in the infected fish. Clinical disease outbreaks are triggered by fish handling operations which cause stress in the animals. Stress induce massive increase in bacterial shedding from the carrier subpopulation at the same time as the naïve subpopulation is susceptible to infection and development of clinical yersiniosis. Addition of Y. ruckeri specific phages to fish holding water during such handling operations can effectively control bacterial infection pressure in the water, and through this mechanism prevent triggering of disease outbreaks.

Successful development of safe and efficacious bacteriophage products for antibacterial use demands that the developer complies with 1) the fundamental laws of nature, 2) the evolving laws of markets, and 3) the man-made laws governing quality, safety and efficacy of various product classes.

Phage host specificity put great restraint on market value for each product. The cost of product development, registration and maintenance is therefore pivotal for the development of phage- based antibacterials.

Finding the right balance between 'medicinal viability' (including quality, safety and efficacy) and 'commercial viability' for phage-based antibacterials will require close and transparent collaboration between academic institutions, industry stakeholders, and the relevant authorities.

Biosketch

Olga Kolaj-Robin, PhD

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Olga Kolaj-Robin joined the European Pharmacopoeia Department of the European Directorate for the Quality of Medicines and Healthcare in 2015 where she works as Scientific Programme Manager coordinating the work of European Pharmacopoeia Groups of Experts and Working Parties. In particular, she coordinates the activities of Bacteriophages Working Party in charge of elaboration and maintenance of pharmacopoeial texts in this field. She holds a PhD in in Protein Biochemistry from University of Limerick (Ireland) and a MS in Molecular Biotechnology from Gdansk University of Technology (Poland)..

Abstract

Olga Kolaj-Robin

Activities of the European Pharmacopoeia in the field of bacteriophages

Rising levels of antimicrobial resistance, identified as one of the leading threats to global public health and development, have prompted renewed interest in the use of phage therapy for the treatment of bacterial infections. This led the European Pharmacopoeia Commission (EPC) to make the creation of European pharmacopoeia (Ph. Eur.) texts standardising requirements for phage therapy one of its top priorities for the years 2023-2025. The general chapter Phage therapy medicinal products (5.31) published in Supplement 11.6, constitutes the first such text and provides a framework of requirements for the production and control of phage therapy products, simultaneously allowing a degree of flexibility commensurate with the complex approaches currently employed in this emerging and rapidly evolving field.
The work is now focused on creating the general chapter on bacteriophage potency determination.

Biosketch

Hansjörg Lehnherr, PhD

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lHansjörg Lehnherr holds a PhD in molecular biology from the University of Basel (Switzerland). In his thesis he studied regulatory aspects of the life cycle of a temperate bacteriophage. After postdoctoral studies at the National Institutes of Health in the US, where he studied the stability of a plasmid prophage, he held positions at universities in Denmark and Germany, doing basic research, naturally connected with bacteriophages. In 2007 he left the academic world in order to try to transfer the accumulated knowledge of almost 100 years of bacteriophage research into practical applications. Since 2009 he is the chief scientific officer of the Phage Technology Center GmbH in Germany, which focuses on bacteriophage applications in everything but humans. Relevant pathogens are Salmonella, E. coli, Listeria, Campylobacter, Clostridium, Staphylococcus, Streptococcus, Pseudomonas, Vibrio and Aeromonas. The treatment of bovine mastitis had a prominent place in all these efforts and he will report recent developments in this area.

Abstract

Hansjörg Lehnherr

A bacteriophage product against Staphylococcus aureus to treat bovine mastitis

Background and Challenges:
Bovine mastitis is a significant, economical problem in dairy farming. A wide range of bacterial pathogens can cause an inflammation of the udder, but Streptococcus uberis and Staphylococcus aureus are the most relevant pathogens to date. Affected cows are visibly ill and produce milk that contains high numbers of somatic cells. Above a certain threshold the dairy will reject the milk, leaving the farmer with the economic loss. The use of antibiotics is still the standard treatment for mastitis, but the development of antibiotic resistant pathogens (e.g. MRSA) significantly reduces the cure rates achieved by the veterinarians. As a last resort affected cows are prematurely slaughtered, generating further losses for the farmer. Thus, alternative strategies to treat mastitis are in high demand.

Proposed Approach:
The idea to use bacteriophages as an alternative to fight pathogenic bacteria, especially antibiotic resistant bacteria, is not novel. A number of groups isolated bacteriophages against Staphylococcus aureus, with the intention to treat bovine mastitis, but only few studies made it past the laboratory concept stage. Some of the problems were associated with the stability and activity of the bacteriophages in the complex medium "raw milk". Others with the purity, formulation and route of application.

Conclusions:
We report the development of a bacteriophage product with a broad range of activity against Staphylococcus aureus strains known to cause mastitis. We show that this product is active in raw milk and is able to cure bovine mastitis in lactating cows. Aspects about the regulatory status and the economic relevance of such a product will also be addressed.

Biosketch

Bryce L. Lunt, PhD

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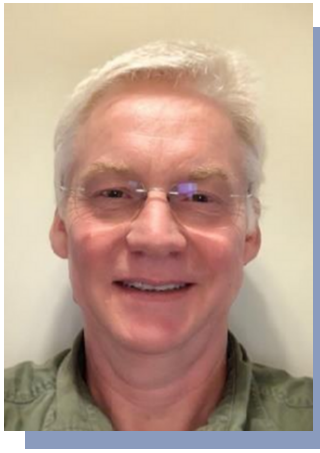
Dr. Bryce Lunt holds a PhD in Biological Sciences from the University of Pittsburgh. Dr. Lunt is currently a Principal Scientist at Zoetis, specializing in Anti-Infectives. At Zoetis, Dr. Lunt contributes to the Antibacterial Susceptibility Surveillance Team and oversees in vitro lab testing of novel antibacterial candidates. Over the past 16 years, he has been involved in various phage-related initiates, including the HHMI SEA-PHAGES program and phage hunting to treat American Foulbrood and Fireblight. Additionally, Bryce leads a team focused on phage discovery.

Biosketch



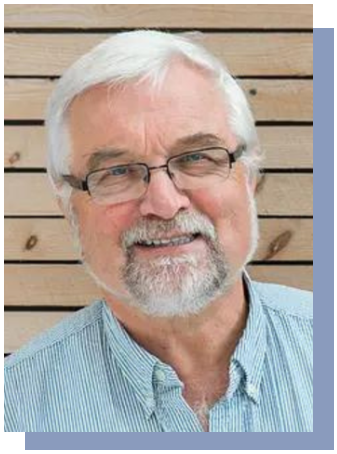
David Mackay

Advisor to the Board

UK

David qualified as a veterinary surgeon andworked in generalveterinary practice before obtaining postgraduate degrees in immunology from the Universities of Birmingham and London. He then embarked on a research career in exotic viral diseases of livestock culminating as Head of the Pirbright Laboratory of the Institute for Animal Health.David subsequently moved into the regulatory area, assuming the posts of Head of Immunologicals and then Director of Licensing at the Veterinary Medicines Directorate in the UK before moving to the European Medicines Agency as Head of Veterinary Medicines. During his career he haspublished widely on epizootic diseases of livestock and on regulatory issues, particularly in relation to veterinary vaccines. David retired from the EMA in 2018 and now provides independent advice to governmental and non-governmental bodies in relation to regulation and use of veterinary medicines.

Biosketch



Paul J. Midtlyng, DVM, Ph.D

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Since 1983 I have been working to support the control of infectious diseases in salmonid farming: designing zoo-sanitary strategies, documenting the effect of monovalent and multivalent adjuvanted bacterial vaccines, and studying genetic susceptibility and selective breeding to increase disease resistance. I pay great interest in the novel approach of using bacteriophages to reduce overgrowth and resultant infection pressure arising from facultatively pathogenic bacteria under aquaculture conditions.

Biosketch

Dušan Palić, DVM, PhD, Dipl. ECAAH

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Professor Palić comes from a long line of veterinarians and educators, being the third generation Professor of Veterinary Medicine. He received D.V.M. and MVSc degrees from Faculty of Veterinary Medicine in Belgrade, Serbia, and Ph.D. from Iowa State University College of Veterinary Medicine. Dušan is a founding member, certified aquatic veterinarian (CertAqV), and Past President of World Aquatic Veterinary Medical Association (www.wavma.org). He is a founding diplomate and former Vice-President of European College of Aquatic Animal Health (ECAAH, www.ecaah.org), and the Director of the International Aquatic Veterinary Biosecurity Consortium (IAVBC, www.iavbc.org). Dušan is chairing the European Medicine Agency workgroup for evaluating medicinal substances and is an expert member in WOAH workgroup to provide guidelines for antimicrobial use monitoring in aquaculture. His daily work as Chair for Fish Diseases at LMU Munich includes research, teaching, diagnostic, and extension services.

Biosketch

Jean-Paul Pirnay, PhD

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Graduated as Industrial Engineer in Biotechnology at the University College Ghent and obtained a Scientific Degree in Agriculture Development at the University of Ghent. Performed his military service in 1993 and served since then as a researcher in the Queen Astrid Military Hospital (QAMH) in Brussels, where he contributed to the development of the human cell and tissue banks and to the introduction of molecular microbiology and bacteriophage therapy. Received a PhD in Medical Sciences from the Vrije Universiteit Brussel in 2003. He is currently head of the Laboratory for Molecular and Cellular Technology (LabMCT) of the QAMH and is involved in several research projects, such as the synthetic production of bacteriophages. He participated in several National, EU and NATO research task groups and evaluation/advisory committees and (co-)authored ~130 peer reviewed scientific publications (>10.000 citations, h index: 57)

Biosketch

Johan Quintens

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Johan Quintens is Chief Scientific Officer at Vesale Bioscience.
Johan holds a Master Degree in Biological Sciencesfrom the Rijks Univeriteit of Ghent (RUG) with specialization in Bacteriology, Endocrinology and Parasitology.He also obtained a post-university Masters in Business Management at UFSIA (University Faculties Sint-Ignatius Antwerp).

Johan has a career of more than 30 years in the pharmaceutical industry.
He started as researcher in beta lactam antibotics, then acted as marketeer at Yamanouchi Pharma and spent 13 years as Managing Director at Tramedico. In 2004 he launched his own management and development company in Biotechnology and Health care.
Johan joined Vesale Pharma about 20 years ago, coming back to its primary interest in microbiology. He developed the company's current product range of probiotic products and formulated the probiotic microencapsulation technology which is at the basis of the International success of the company. He was also present since the start of the Bacteriophage project and developed the phagogram and the basis of the actual phage business of Vesale Bioscience.
Johan is the author of 8 patents in probiotic and phage technology.

Abstract

Johan Quintens

Phage therapy for veterinary applications from the perspective of a manufacturer

Vesale Bioscience is a Biotech Pharmaceutical company dedicated to the development of phage therapy products for human medicinal applications. The company developed a scalable model for a personalized approach of phage therapy. The success of a phage therapy intervention highly depends on the correct choice of the phages to apply. To perform this determination correctly we developed an automated susceptibility test which can be performed in each hospital laboratory. This automatic phagogram can detect the activity of upon 38 phages simultaneously in less than 4 hours and is the cornerstone of the therapeutic model. Once the right phages detected, a personalized cocktail can be formulated by the hospital pharmacist.

We applied this method in a pilot project on cow mastitis where the individualization of the therapy is done on a farm level. The automated phagogram is first used to develop the phage cocktail on basis of a single case of mastitis, postulating that future cases in the same pharm will have the same pathogen as origin.

By developing this project, several considerations and specific hurdles to overcome, typical for the veterinary approach of phage therapy, came up. These are as well of technical, regulatory as commercial nature and are prone to the nature and specific character of phages and phage therapy.

As a conclusion: we have a clear picture and pathway to develop human phage therapy products, but the models we developed and are successful in human therapy cannot be transposed directly to a veterinary situation. Questions like individualization of the therapy, concomitant use of antibiotics or not and others will need to be answered before phage therapy can be supplied with success on difficult-to-treat infections in veterinary.

Biosketch

Prof. Damien Thiry

ULiège, Belgium
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Damien Thiry is Professor of Veterinary Bacteriology at Liège University, Belgium. He obtained a PhD thesis on the interactions between suids and hepatitis E virus in the Virology unit of the FARAH Research Center (Faculty of Veterinary Medicine, ULiège) in collaboration with the Scientific Institute of Public Health in Brussels (Sciensano). Simultaneously, he performed a master degree in Veterinary Public Health – Emerging Diseases. He is Diplomate of the European College of Veterinary Microbiology since 2019 and Director of the Belgian Residency Center for this College. He is working since 2014 in the Bacteriology team of the veterinary faculty. He performed two post-doctoral research stays: one in Pasteur Institute (Paris) and a second one at KULeuven where he developed a Galleria mellonella model of phage therapy against Klebsiella pneumoniae. His main research topics are the study of antimicrobial resistance mechanisms and the study of bacteriophages as potential treatment against bacterial infections.

Abstract

Damien Thiry

Phage therapy in companion animals: which way for an efficient treatment?

Phage therapy has garnered increasing interest as an alternative or complementarity to conventional antibiotics, particularly in the context of antimicrobial resistance (AMR). This presentation will explore the potential of phage therapy for managing bacterial infections in companion animals. Companion animals encompass dogs and cats but also other animals such as rabbits, horses, rats, birds. The close contacts between these animals and Human and the sharing of bacteria from their microbiota, including the exchange of AMR genes, make it essential to develop efficient treatment against MDR bacteria but also to pay attention to the resistance that could occur against these new treatments. Even if cases are reported, the literature on phage therapy in companion animals is scarce but transposition from human medicine can easily be made in this context. This talk will present a series of cases going through otitis or uterine infection in dogs to osteoarticular implant infection in cats and cutaneous infection in horses.

Biosketch

Etienne Thiry

Liege University
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Etienne Thiry was born on the 4th of April 1957 in Etterbeek (Brussels). He was graduated as doctor in veterinary medicine in 1980 and in veterinary sciences (PhD) in 1985. He is retired diplomate member of the European College for Veterinary Public Health. From 1st October 2022 he is ordinary professor emeritus of veterinary virology and viral diseases, Liège University, Belgium. He is also professor emeritus of veterinary virology at the Free University of Brussels. He won the International Pfizer award by the international committee of the World Buiatrics Society in 1996. He won the Gaston Ramon award by the French Academy of Veterinary Medicine in 2008. He was recognized by the European Society for Veterinary Virology as honorary member in 2009. He received the award Prix de la Francophonie by the Fédération des Associations francophones des vétérinaires d'Animaux de Compagnie (FAFVAC) in 2011. The French Veterinary Technical Groups (GTV 52) awarded him the Jean-Jacques Tondreau Excellence Prize in 2012.

Etienne Thiry is member of the Royal Academy of Medicine of Belgium. He is also member of the National Committee for Microbiology of the Belgian Royal Academy. He is vice-chairman of the Veterinary Biologicals Committee of the International Alliance for Biological Standardization.

Biosketch

Etienne Thiry

Liege University
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He is consulting member, previously acting- and vice-chairman, of the European Advisory Board on Cat Diseases. He is formze vice-chairman of the board of directors of the federal scientific institute Sciensano (2018-2024); former chairman of the scientific committee at the Belgian Food Safety Agency (Afsca) (2014-2021); former chairman of the Risk Assessment Group Covid Animals (2020-2021); former chairman of the expert committee for animal health and welfare at the French Agency for Food, Environmental and Occupational Health and Safety (Anses) (2012-2018). He was also chairman of the board of directors of the non-profit association Formavet active in veterinary continuing education.

His research interests cover several aspects of animal virology, especially the study of animal virus-host interactions and the evolution of viral populations in noroviruses and hepeviruses; in applied research, virucidic activity and the quality of personal protective equipments, especially in the context of the Covid-19 pandemic. These scientific activities generated more than 500 papers in specialised scientific journals (h-index Scopus 48). He is the author of the four books in the collection Clinical Virology at the Editions du Point Vétérinaire on clinical virology of ruminants, dog and cat, swine and equids.

Biosketch

Bruce Thomsen, DVM, PhD, DACVP

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Dr. Thomsen is the Virology Section Leader within the Policy, Evaluation and Licensing Unit at the United States Department of Agriculture, Center for Veterinary Biologics. His role is to evaluate new and existing veterinary biologics. Prior to moving to CVB in 2016, he was a pathologist at the National Veterinary Services Laboratories from 2001 to 2016. He received his PhD from Iowa State University in 2001 following 7 years of clinical practice and obtained his DVM from the University of Missouri-Columbia in 1990.

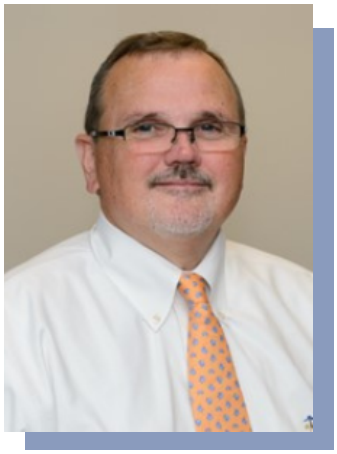
Abstract

Bruce Thomsen

x

x

Biosketch



Jeffrey L. Watts, PhD

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Dr. Jeffrey L. Watts currently holds the position of Research Director, Anti-Infectives for Zoetis, Inc. Dr. Watts has had a varied career over the past three decades that has spanned both clinical and veterinary microbiology. He has been active in both human and animal health antibacterial discovery programs over the past three decades where he has contributed to the development of ceftiofur, clindamycin, pirlimycin, spectinomycin, cefpodoxime, and linezolid. Jeff leads programs in the Alternatives to Antibiotics space including phage, antimicrobial peptides, immunomodulation, and anti-virulence. Jeff was the founder of the Clinical and Laboratory Standards Institute (CLSI) Subcommittee on Veterinary Antimicrobial Susceptibility Testing. Jeff received his PhD from Western Michigan University and has published over 100 articles in scientific journals.

Abstract

Jeffrey L. Watts

The Importance of CMC for Bringing Animal Health Phage Products to Market

The ability to produce a consistent, stable pharmaceutical product through a well-defined, robust manufacturing process is a key regulatory component. The Chemistry, Manufacturing, and Control (CMC) component of the regulatory dossier is essential for regulatory approval and eventual marketing of therapeutic agents including phage-based products. If the phage product is a cocktail, the CMC section will need to define the production specifications for each phage as well as the process for combining the phages to produce the final product. Early development of the CMC process is essential as regulatory agencies often require that pivotal studies be performed using product produced using the final process. The CMC section is also essential for establishing the final Cost of Goods (CoGs) for the product which will affect commercial viability. Given the potential for contamination of phages In both vaccine and pharmaceutical products, standalone GMP manufacturing facilities will be required for phage production. This is a substantial capital investment with costs generally in the 15-25M USD range. Moreover, capacity of those manufacturing facilities could be very large depending on the indication. For example, a poultry product would require a very large number of doses to treat a significant portion of the global poultry population (~14B). In conclusion, manufacturing considerations may be a considerable hurdle to the successful introduction of phage-based products. Fully understanding the market needs and cost structures may allow these hurdles to be overcome.

POSTERS

Exploiting *Campylobacter jejuni* phage receptor binding protein for rapid and specific bacterial detection

Su Zar Chi Lwin[1], Yoshimitsu Masuda[2], Ken-ichi Honjoh[2], Takahisa Miyamoto[2]
[1]Graduate School of Bioresource and Bioenvironmental Sciences,[2]Faculty of Agriculture, Kyushu University, Fukuoka, Japan

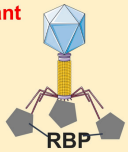
BACKGROUND

Campylobacter jejuni

- Gram-negative microorganism
- Foodborne pathogen, causes neuropathy conditions such as Miller-Fisher and Guillain-Barre syndromes

Early and accurate detection is as important as treatment strategies

Phage Receptor Binding Protein (RBP)
- Attachment apparatus
- Major protein that ensure host specificity
- Promising bacterial detection tool



Utilization of RBP for rapid detection of *Campylobacter*

RESEARCH FLOWS

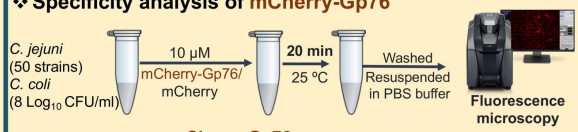
❖ Synthesis, Expression and Purification of chimeric protein

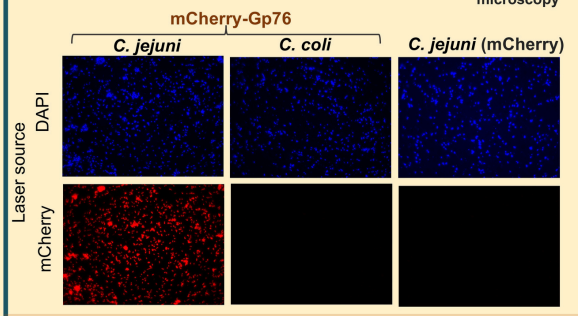
Putative RBP- **Gp76** – 1032 bp
Protein id –QVW54049.1

Deduced molecular mass ~67 kDa

mCherry-Gp76 and mCherry were successfully expressed and purified

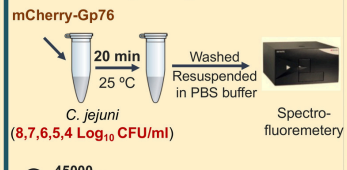
❖ Specificity analysis of mCherry-Gp76

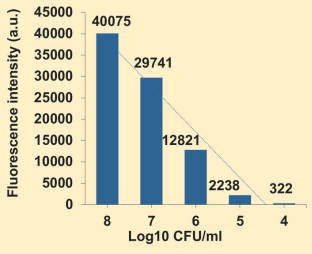




Fluorescence microscopy images: Specifically bound to *C. jejuni*. mCherry-Gp76 displayed the broader host range (92 %,46/50 strains) compared to its parent phage vB_CjeM-PC10 (62 %, 32/50 strains).

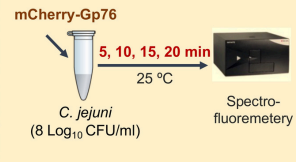
❖ Sensitivity analysis

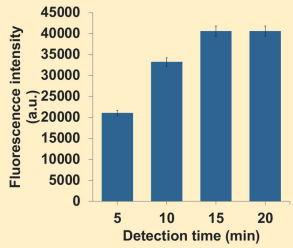




High sensitivity level: Detectable from minimum infectable concentration.

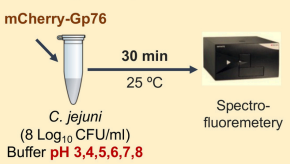
❖ Detection time determination

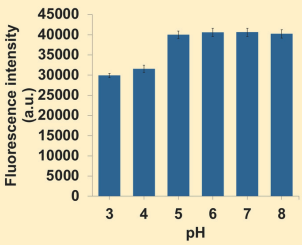




mCherry-Gp76 displayed the shortest detection time from 5 min.

❖ Stability analysis

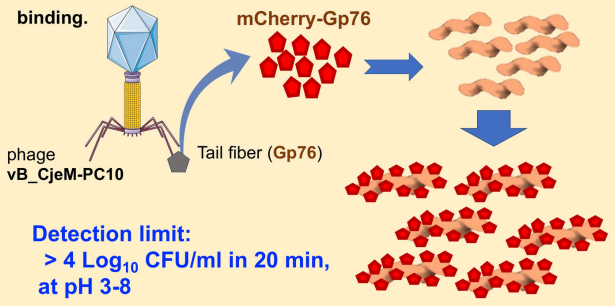




Detectable in various pH level that is ideal range of chicken intestine.

CONCLUSION

- **This study aimed to identify RBP of *C. jejuni* phage for specific bacterial binding.**

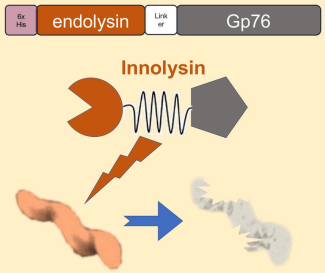


Detection limit:
> 4 Log_{10} CFU/ml in 20 min, at pH 3-8

RBP Gp76 was validated as specific, sensitive and rapid detection biomolecule for accurate prevention of *C. jejuni* outbreaks.

FUTURE DIRECTION

- **Construction of Innolysin (Fusion of receptor binding protein and lysin)**



Bactericidal against *C. jejuni* without resistance emergence

In vitro efficacy and individual bacteriophage treatment of *P. aeruginosa* in dogs

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Introduction

Multi-drug resistant (MDR) and often zoonotic bacteria are a major problem in human and veterinary medicine posing a global threat to public health. The fight against antimicrobial resistance (AMR) is conducted in terms of the antimicrobial stewardship. Bacteriophages as an alternative or additive to antibiotics have the potential to counteract this problem. The aim of this study was to determine the *in vitro* efficacy of bacteriophage cocktails, commercially available in Georgia for use in humans, as well as a veterinary bacteriophage suspension against bacterial isolates from dogs and to accompany an initial case study of otitis externa caused by *Pseudomonas aeruginosa*.

Material and Methods

Species identification focusing on bacterial field isolates of *P. aeruginosa* from dogs was performed using Maldi-ToF MS (Bruker Cooperation, USA). Antimicrobial susceptibility tests (AST) were obtained using Micronaut Systems according to CLSI guidelines with resistance to at least three antimicrobial groups. The susceptibility of the bacterial isolates was tested by phagogram according to standard procedures established at Eliava Institute. Bacteriophage cocktails from Eliava BioPreparations and a bacteriophage suspension (VetPS) from Eliava Institute were tested. Lysis zones were scored as positive (S); absent lysis zones as resistant (R). In addition, the bacteriophage titer was determined by dilution series. The bacteriological monitoring during the case study included cuture, indentification by Maldi-ToF and AST and was performed by Laboklin GmbH & Co. KG.

Results and Discussion

In vitro efficacy of bacteriophage cocktail “Pyo”, “Intesti” and a veterinary PS (VetPS)

A lytic activity of commercially available bacteriophage cocktails “Pyo” and “Intesti” for use in humans and of a VetPS could be demonstrated against bacterial isolates of dogs. Depending on the different batch of the bacteriophage cocktails (stated by batch numbers), overall a good efficacy against *P. aeruginosa* was observed **(Fig. 1)**. For “Pyo” M1-1202 an *in vitro* efficacy of 80 % compared to “Pyo” M1-1301 of 76 % show slightly differences. In contrast to the bacteriophage cocktail “Intesti” M2-1202 with an *in vitro* efficacy of 83 % differs to “Intesti” M2-1301 with an efficacy of only 69 %. Differences between the batches have been observed due to the regular adaption of the bacteriophage cocktails to human isolates. However, the VetPS, which was adapted to animal derived isolates, shows an *in vitro* efficacy of 99 %.

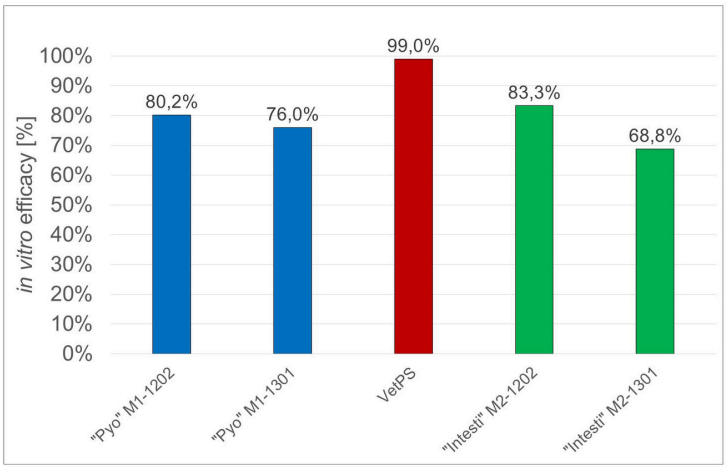

Fig. 1: *In vitro* efficacy of two commercially available bacteriophage cocktails with different batch numbers and one veterinary bacteriophage suspension (VetPS) against *P. aeruginosa* from dogs [n=96] were tested.

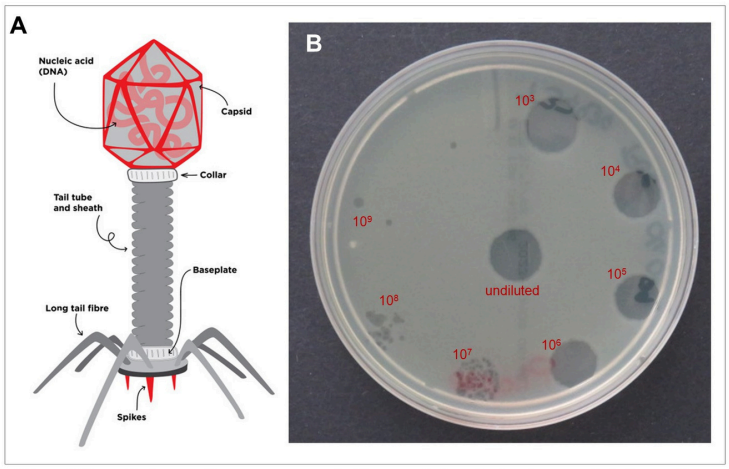

Fig. 2A.: Schematic representation of bacteriophage (picture from Phagenzentrum). **2B.: Phagogramm (dilution series)** of VetPS against *P. aeruginosa* (isolated from otitis externa, dog), Titer amounts 10^9 pfu/ml.

Case study of a dog with otitis externa caused by *P. aeruginosa*

A dog (irish setter, 5 years old) suffering from purulent, painful, recurrent otitis externa caused by *P. aeruginosa* had limited treatment options due to intrinsic and acquired resistances of *P. aeruginosa*. Multiple and prolonged antibiotic therapies (amoxicillin and clavulanic acid, marbofloxacin, enrofloxacin, florfenicol) were unsuccessful. Thus, therapy emergency was given as well as the veterinary indication for not licensed treatment to avoid unacceptable suffering of the animal (Article 112, EU reg. 2019/6). A bacteriophage therapy was carried out by the treating veterinarian including thorough cleaning of the ears to remove secretions and cell detritus prior to application of the bacteriophages. Specific local bacteriophage treatment was carried out 5 consecutive days (5 ml, 2 times a day) **(Fig. 2B),** followed by 5 days of local antibiotic therapy (gentamicin). The treatment was successful with no recurrent otitis during time of clinical observation (9 months).

Conclusion

This is the first preliminary study investigating the *in vitro* efficacy of commercially available bacteriophage cocktails from Eliava BioPreparations and a veterinary bacteriophage suspension (VetPS) from Eliava Institute against bacterial isolates. The five days treatment of a dog with bacteriophages followed by five days treatment with antibiotic finally cured the infection. This case demonstrates that a meaningful combination of phage and antibiotic therapy is useful to combat infections with MDR bacteria also in the manner of One Health.

Acknowledgement

Mzia Kutateladze from Eliava Institute of Bacteriophages, Tbilisi, Georgia, and Vakhtang Pavlenishvili from Eliava BioPreparations, Tbilisi, Georgia

Identification of effective bacteriophages for mastitis prevention in goats

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International Livestock Research Institute. Nairobi, Kenya

BACKGROUND

Kenya, goat farming and phages

1 Most abundant livestock
- 28 millions goats
- 297 millions liters of milk/year

2 Household management
- > 2 millions households, managed by women
- Referred as **mobile bank**

3 Health threat
- Mastitis caused by *Staphylococcus* sp
- Impact on production and economy

4 What we propose
- **Lytic bacteriophages** to prevent mastitis infections

METHODOLOGY

- Host range of 14 staphylococcal phages from Ulaval collection over 93 *Staphylococcus* strains :
  - Bacterial lysis on agar plates or not?
  - If yes, Efficiency of plating (Khan Mirzaei & Nilsson, 2015)

RESULTS

- Sinhovirures have very narrow host range on tested strains
- Podoviruses (P68, 44AHDJ), large host range on S. aureus strains
- Myophages, mostly Kayvirus (RuSa1, K, 812, Stab21) and Silviavirus (Remus) have the largest host range, both on *S. aureus* and non-*aureus* strains.
- **RuSa1, K, 812 and Stab21** produced new virions on non-pathogenic strains *S. xylosus* and *S. carnosus*.

ACKNOWLEDGEMENT

Funded by IDRC · CRDI Canada

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FAO, 2017 – Africa Sustainable Livestock 2050
Khan Mirzaei, M., Nilsson, A.S., 2015. PLOS ONE 10, e0118557.

phage.ulaval.ca

RESULTS

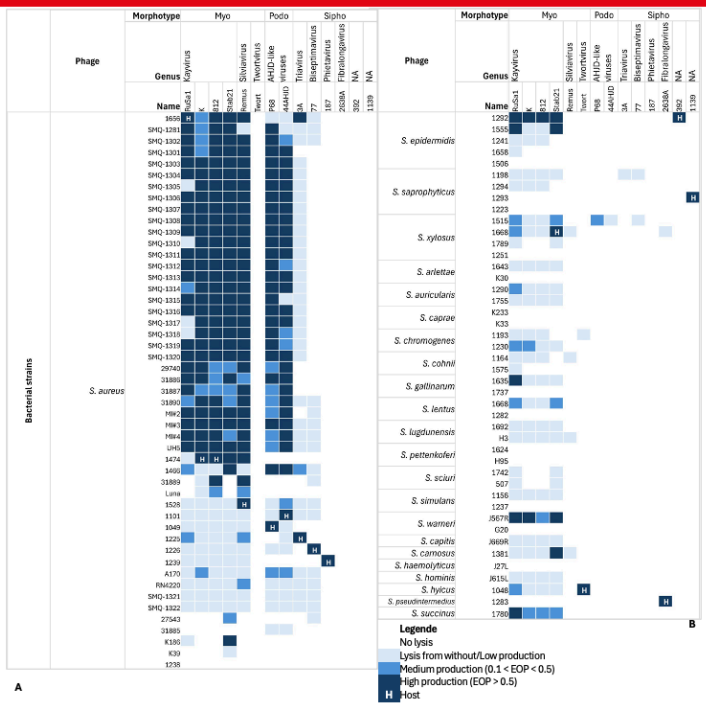


Fig: Host range of 14 phages over *Staphylococcus aureus* (A) and non-*aureus* strains (B)

Conclusion

- **RuSa1, K, 812 and Stab21,** best candidates for *Staphylococcus* sp control
- Future research exploring these phages in combination therapies

Alphagos *Vibrio*, a tailored phage cocktail reduces *Vibrio parahaemolyticus* infection in Indonesian shrimp hatcheries

Agniah[2], Marine Feyereisen[1], Ilias Theodorou[1], Dony Bayu Pamungkas[2], Refiana Lestary[2], Romain Sausset[1], Mayumi Suzuki[1], I Wayan Wisaksana Yasa[2], Inna Herliana[2], Adèle James[1]

Introduction

Vibrio parahaemolyticus infection is a major threat to **whiteleg shrimp (*Litopenaeus vannamei*) aquaculture**, causing significant shrimp mortality and leading to considerable economic losses for hatcheries and farms. Bacteriophages provide a promising antibiotic alternatives due to their high efficacy and specificity towards target bacteria. **phagos** and **Vaksindo** Satwa Nusantara develop bacteriophage products for aquaculture. This experiment aims at determining the efficacy of bacteriophage to eliminate *Vibrio parahaemolyticus* bacteria in the maintenance environment of vannamei shrimp.

Objective

Show the robustness of **Alphagos** *Vibrio* phage cocktail by treating naturally *V. parahaemolyticus*-infected shrimp larvae sampled from an Indonesian hatchery with a phage cocktail designed to target *V. parahaemolyticus* strains isolated from other hatcheries in Indonesia.

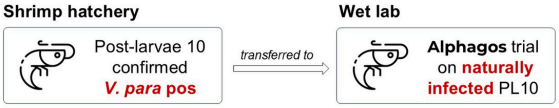
Cocktail design

Designed to target 13 representative strains (but not tested on the strains infecting the shrimp larvae at the moment of the trial)
→ isolated from 5 **Indonesian shrimp hatcheries**, from 3 islands, across more than 3000 km.

Prediction *in silico* of the best phage cocktails to test from **phagos** phage collection
Prediction further confirmed *in vitro on the 13 representative strains*
→ selection of a **5-phages cocktail**

Trial design

Shrimp origin



Trial conditions

Condition	**Alphagos** *Vibrio*
A	positive control
B	10^3 PFU/mL
C	10^5 PFU/mL
D	10^7 PFU/mL

- No experimental infection as PL10 naturally infected
- 3 biological replicates/condition
- Total *Vibrio* count on Chromagar Vibrio and TCBS
- *V. parahaemolyticus* count on Chromagar Vibrio

Results

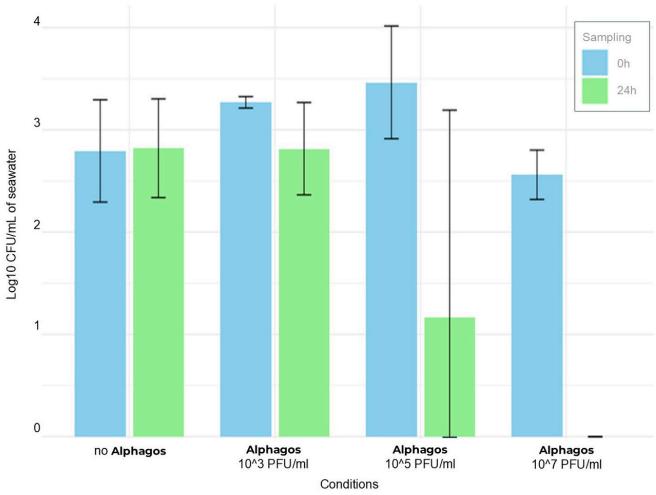


Figure 1 - Mean *Vibrio parahaemolyticus* CFU counts represented in log scale at time 0h and 24h of the trial. Bars represent the mean CFU counts/replicates (x3) and the error bars represent the standard deviation. CFU counts were done on seawater sampled from the trays at time 0h corresponding to the beginning of the trial and after a 24h incubation with/without phage treatment. In the positive control, there is no *V. parahaemolyticus* load difference between time 0h and 24h. When administered at 10^3 PFU/ml, **Alphagos** *Vibrio* does not induce a significant *V. parahaemolyticus* loads reduction in 24h while higher doses, at final concentrations of 10^5 and 10^7 PFU/mL of seawater, led to a **significant reduction** (2 replicates/3 were *V. para* negative) and **total elimination** respectively. **Alphagos** *Vibrio* had no effect on the total Vibrio count (data not shown).

Key takeaways

- **Alphagos** *Vibrio* eliminates *V. parahaemolyticus* from naturally contaminated seawater.
- **Alphagos** *Vibrio* has no effect on the non *V. parahaemolyticus Vibrio* species.
- **Alphagos** *Vibrio* is efficient against a large spectrum of *V. parahaemolyticus* strains isolated in diverse shrimp hatchery hot spots in Indonesia, making it a great product to decontaminate stocks before the farming phase and to reduce the risk of *V. parahaemolyticus*-caused production losses.

[1] Phagos SAS, Paris, France; [2] PT Vaksindo Satwa Nusantara, Indonesia

Therapeutic efficacy of bacteriophage cocktail against multidrug resistant *Streptococcus agalactiae* infection in *Oreochromis niloticus*

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2. **Department of Aquatic Animal Health Management, Faculty of Fisheries Science, Kerala University of Fisheries and Ocean Studies (KUFOS) Ernakulam, Kerala, India. E mail: safeena.mp@kufos.ac.in**

Background

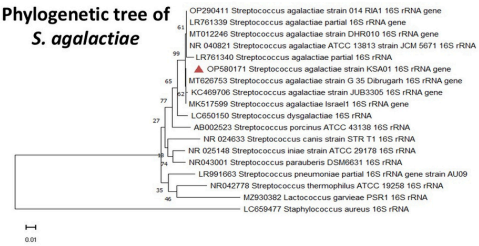
- In aquaculture, **bacterial infections** continue to be a serious economic issue around the world.
- Among the bacterial infections, Streptococcosis caused by ***Streptococcus agalactiae*** is a major one affecting aquaculture worldwide.
- Propelled by the overuse and abuse of **antibiotics** in the aquaculture systems, there is a wide spread of **antibiotic resistant bacteria** in both human and environment, reaching through food chain and recreational activities.
- Aquaculture systems have been identified as the hotspots for **multi-drug resistant (MDR)** bacteria and **antimicrobial resistant (AMR) genes** due to the extensive and indiscriminate use of antibiotics against bacterial pathogens.
- In this scenario, the current aquaculture sector is in urge for an alternate **biocontrol agent**, which helps in supporting the fish health and a healthy environment.
- **Bacteriophage therapy** is a promising alternative strategy to antibiotic resistance and is getting more magnetism due its abundance, host specificity and self-limiting ability
- However, one of the obstacles to phage therapy is **phage resistance**, and it can be acquired through genetic mutations, followed by consequences of phenotypic variations
- **Phage cocktail** composed of two or more bacteriophages can be used for broader spectra of activity and to achieve effectiveness under a greater diversity of conditions and to suppress the emergence of anti-phage resistance in bacteria.
- This study aims to formulate a phage cocktail of five lytic phages (previously isolated and characterize d phages) and to evaluate its **therapeutic potential (*in vivo*)** against *Streptococcus agalactiae* in tilapia (*Oreochromis niloticus*).

Methodology

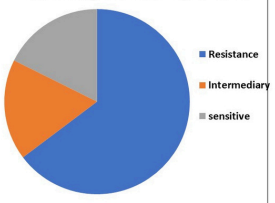
1. **Isolation and molecular confirmation** of *S. agalactiae* from infected tilapia farm, Kerala, India
   - Screening of **antibiotic susceptibility** of *S. agalactiiae* (CLSI, 2021)
2. **Isolation and purification of bacteriophage** against *S. agalactiae* (Adams, 1959)
   - Primary host *Streptococcus agalactiae* KSA/01 (Genbank Acc. OP580171)
3. **Characterization**
   - Growth kinetics- one step growth curve, thermal and pH stability tests
   - Morphology- High resolution Transmission Electron Microscopy (HR-TEM)
   - Genetic diversity- Random Amplified Polymorphic DNA Fingerprinting- RAPD (Shivu et al., 2007)
4. ***In vitro* lytic potential of phages-** monophage and phage cocktail- Turbidity reduction method (Kusradze et al., 2016)- UV-Vis Spectrophotometric measurement (600 nm)
5. **Challenge experiment in tilapia** (*Oreochromis niloticus*) (Khalifa et al., 2018)
   - Fish control (Group A), Bacterial control (Group B), Phage therapy (Group C), Phage control (Group D)
   - Bath therapy- 200 µl/l water
   - (Ethical clearance- 174/ac/08/CPCSEA)
6. **Relative Percent Survival (RPS)**= [1 − (% mortality in test group/% mortality in control group)] × 100

Results

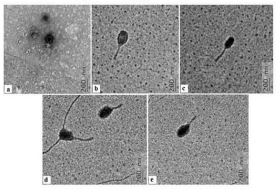
Phylogenetic tree of *S. agalactiae*



Antibiogram of *S. agalactiae*

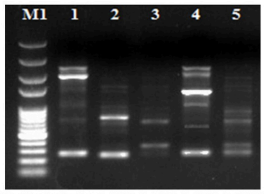


Morphology- HR-TEM



a- SAP1, b- SAP2, c- SAP3, d- SAP4, e- SAP5

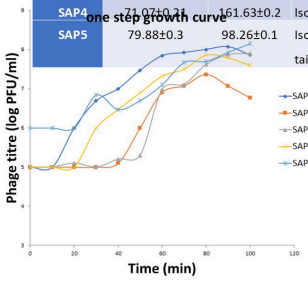
Genetic diversity- RAPD Fingerprinting

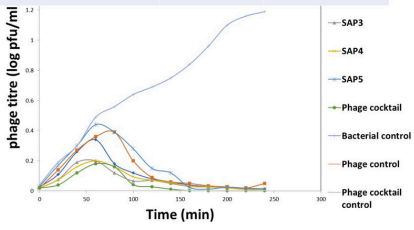


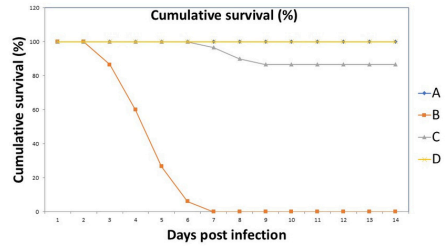
Lane: M1- 1kb plus Ladder, 1- SAP1, 2- SAP2, 3- SAP3, 4- SAP4, 5- SAP5

Phage	Transmission electron microscopy (TEM)				
	Head diameter (nm)	Tail length (nm)	Properties	Family	Order
SAP1	101.52±2	62.60±1	Isometric head and non-contractile tail	Myoviridae	Caudovirales
SAP2	83.34±0.2	128.73±0.1	Isometric head and contractile tail	Siphoviridae	Caudovirales
SAP3	66.61±0.01	152.03±0.1	Icosahedral head and contractile tail	Siphoviridae	Caudovirales
SAP4	71.07±0.21	161.63±0.2	Isometric head and contractile tail	Siphoviridae	Caudovirales
SAP5	79.88±0.3	98.26±0.1	Isometric head and non-contractile tail	Myoviridae	Caudovirales

one step growth curve







Challenge Experiment in *S. agalactiae* infected *Oreochromis niloticus*

Relative percent survival (%)- 86.68%

Conclusion

- Phage therapy, an alternative therapeutic measure is effective in controlling the bacterial infection and it will be **an effective and safe strategy** with **zero impact on both fish health and environment**.
- In this study, **five genetically diverse phages** were formulated as a **phage cocktail** against multidrug resistant *S. agalactiae*. In challenge study, the bacteria got eliminated from the host animal with a **significant reduction in mortality** in comparison with bacterial control
- Thus phage cocktail is found to be an effective therapeutic strategy to control the *S. agalactiae* infection in tilapia. In addition, phage cocktail was found to be safer as it provide **no possibility for the development of phage resistance in bacteria**.

Acknowledgements

1. We acknowledge Faculty of Fisheries Science for providing microbiology laboratory facility of the department of Fish Processing Technology, Kerala University of Fisheries and Ocean Studies (KUFOS), Kerala, India.
2. We acknowledge Sophisticated Test and Instrumentation Centre (STIC), a joint venture of KSCSTE and CUSAT, Cochin, for their help to do HR-TEM analysis of bacteriophages

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RIME High performance genome annotation for a safer and faster-developing phage therapy

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2: Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Canada

INTRODUCTION

Phage therapy is a great opportunity to control multi-drug resistant bacteria. Before releasing large phage amounts in the environment or in patients, caution and regulation demands understanding what genes we might spread.

"High-quality gene finding and functional annotation are vital for successful discovery of new phage-based therapeutics."
– Kutter et al, 2015

"Lamentably, [...] as phage sequencing becomes more high-throughput and automated, we are observing a significant increase in problems"
– Turner et al 2021

2 x 10⁵ [...] contaminated sequences were detected in GenBank. This single most surprising finding was the presence of a piece of a bacterium, in [...] the current version of the human reference genome."
– Steinegger, 2021

IN GENBANK, 70-80% OF GENES OF THE AVERAGE PHAGE GENOME ARE HYPOTHETICAL PROTEINS

MATERIAL & METHODS

« Can automatic genome annotation compare with manual approaches ? »
- Kieran Furlong, University of Ottawa at the 2023 Evergreen Conference

27 Phage genomes from uOttawa's SEA-PHAGES program

Novel annotation with RimeTOOLS 2 – automatic version

Compare structural and functional annotation results

Gene function annotation theory relies on a simple algorithm:

1. Finding a gene that was already annotated using reliable methods.
2. Comparing the gene to annotate to the already known gene.
3. Deciding if the two compared genes are close enough to be considered functional homologs.

As a researcher is selecting a gene function reference database, the choice is twofold:

- Using a small, high-quality, manually curated database like Swiss-Prot
- Using a large, low-quality, uncurated database like NCBI Nucleotide

RimeTOOLS2 uses large, curated custom databases to provide high-quality annotations. It can be configured to add manual curation at every analysis step.

RESULTS & CONCLUSION

	SEA PHAGES *With rTOOLS2 as a reference*	RIME *With SEA PHAGES as a reference*
STRUCTURAL ANNOTATION	+1.5 genes detected / genome	-1.5 genes detected / genome
FUNCTIONAL ANNOTATION	-4.2 better annotated genes / genome	+4.2 better annotated genes / genome

RimeTOOLS2's automatic annotation is getting close to manual annotation. However, a state-of-the-art result can only be obtained when combining both approaches.

It is important not to blindly rely on software : RimeTOOLS2 automatic annotation is suitable for screening a phage collection for genes of interest (lysogeny, virulence or AMR for example).

However, therapeutic phages should be characterized in detail, using manual curation : the health of patients and of the environment is at stake.

Alphagos, a tailored phage cocktail, controls multiple-*Salmonella enterica* strain colonization in broiler chickens

Ilias Theodorou*[1], Marine Feyereisen*[1], Fitri Luthfianti Nur Annisaa[2], Irfan Refangga[2], Houa Oukaci[1], I Wayan Wisaksana Yasa[2], Inna Herliana[2], Adèle James[1]

Introduction

Salmonella enterica is a bacterial pathogen responsible for salmonellosis, a significant public health concern due to its impact on human health. This pathogen is commonly found in a variety of animal-origin products intended for human consumption, with poultry eggs and meat being its main reservoir and the major sources of **human *Salmonella* infection**. Given the emergence of multi-drug-resistant bacteria and the resulting restrictions on antibiotic use in livestock, alternative strategies are needed in the poultry sector to prevent the presence of *Salmonella enterica* in the animals and reduce the risk of infection. In response to this challenge, a tailored phage cocktail has been developed by **phagos** and tested in **Vaksindo** facility, aiming to control *Salmonella enterica* colonization in broilers.

Cocktail design

Designed to target 25 representative strains
→ isolated from **25 Indonesian chicken farms**, from 7 islands, across more than 3000 km.

Prediction *in silico* of the best phage cocktails to test from **phagos** phage collection
Prediction further confirmed *in vitro on the 25 representative strains*
→ selection of a **4-phages cocktail**

Trial design

Trial duration: 21 days

Animals: 4 week-old chickens
10 animals/cage
2 cages/conditions

Bacterial administration: 2 rooms
- room A - 1 *Salmonella enterica* strain
- room B - 5 diverse *Salmonella enterica* strains

Condition	*Salmo* admin	**Alphagos**
A	PO** at d0	
B	PO at d0	PO at d0
C	PO at d0	drinking water x21 days
D		PO at d0
E		

***PO: per os = oral administration*

Results

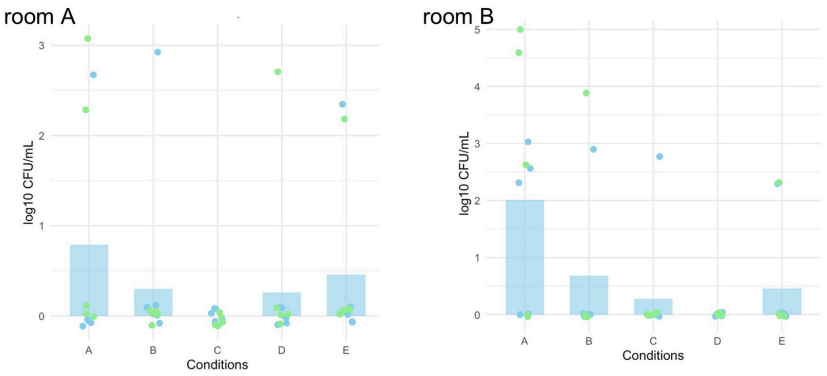


Figure 1 - Mean of the *Salmonella enterica* CFU counts on XLD expressed in log10 CFU/mL of organs resuspended in sterile PBS in a weight:volume 1:1 ratio for each condition of the trial. The bars represent the mean *Salmonella enterica* CFU counts/condition. Each count (5 animals x2 cages) is represented by a dot, each cage is represented by a color (blue and green dots). Birds in room A were administered with 1 *Salmonella enterica* strain while in room B they were coadministered with 5 diverse strains. Both phage treatments PO administration (B) or via drinking water (C) resulted in a decrease in *Salmonella* loads in fecal samples collected over time (data not shown) as well as in the organs collected on day 21 compared to the positive control (A).

When administered in drinking water (C), **Alphagos** phages were **recovered at high titer** throughout the trial in fecal samples and in organs after culling at day 21. When a single shot of **Alphagos** was administered at day 0, active **Alphagos** phages were recovered up to day 15 in fecal samples and in organs after culling (data not shown).

In room A, **no *Salmonella enterica* was detected after 21 days** in the organs when **Alphagos** was administered in drinking water (C), with CFU below the *Salmonella* load in the negative control (E). Similarly, in room B, *Salmonella* load was lower when **Alphagos** was administered in drinking water (C), with CFU below the *Salmonella* load in the negative control (E). Those results indicate that **Alphagos** was effective against strains not initially targeted in the cocktail.

Administration of **Alphagos** in drinking water throughout the trial (C) led to lower *Salmonella* counts in both rooms compared to a single PO shot (B).

Key takeaways

- A single dose of **Alphagos** ensures high recovery rate of phages up to day 15.
- Administration of **Alphagos** through drinking water is optimal as it reduces more efficiently *Salmonella enterica* loads in live birds.
- The *Salmonella* isolates represent the strains present in Indonesia that pose a challenge. **Alphagos**, a four-tailed cocktail, specifically targets the dominant *Salmonella* strains that may be responsible for *Salmonella* infections in humans.
- This study demonstrates the potential of **Alphagos** as an **effective strategy against *Salmonella enterica* in poultry production**.

* These authors contributed equally to this work; [1] Phagos SAS, Paris, France; [2] PT Vaksindo Satwa Nusantara, Indonesia

Alphagos, a tailored phage cocktail, reduces *Salmonella enterica* colonization in chicken carcasses

Marine Feyereisen*[1], Ilias Theodorou*[1], Gusti Ngurah Bayu Krisna Mahardika[2], Febriana Wulandari[2], Dony Bayu Putra Pamungkas[2], Helmy Tamrela[2], Natalie Kuljanishvili[1], Anggita Zain Komala[2], Muhammad Hasdullah[2], Muhamad Furqon Sukrilah[2], Muhammad Reza[2], Faiza Senja Widya Perdana[2], Ragil Gunawan[2], Rama Alfizar[2], Refiana Lestary[2], Inna Herliana[2], Khrisna Putera[2], Adèle James[1]

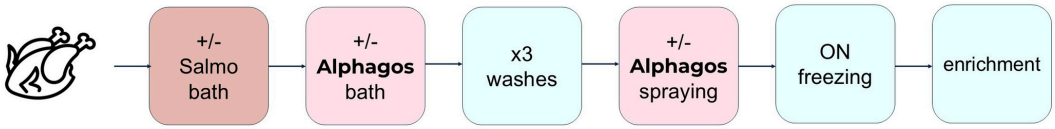
Introduction

Salmonella enterica is a bacterial pathogen responsible for salmonellosis, a significant public health concern due to its impact on human health. This pathogen is commonly found in a variety of animal-origin products intended for human consumption, with poultry eggs and meat being its main reservoir and the major sources of human *Salmonella* infection. Given the emergence of multi-drug-resistant bacteria and the resulting restrictions on antibiotic use in livestock, alternative strategies are needed in the poultry sector to prevent the presence of *Salmonella enterica* in the animals and reduce the risk of infection. In response to this challenge, a tailored phage cocktail has been developed by **phagos** and tested in **Vaksindo** facility, aiming to control *Salmonella enterica* colonization in chicken carcasses.

Cocktail design

Designed to target 25 representative strains → isolated from **25 Indonesian chicken farms**, from 7 islands, across more than 3000 km.
Prediction *in silico* of the best phage cocktails to test from **phagos** phage collection
Prediction further confirmed *in vitro on the 25 representative strains* → selection of a **4-phages cocktail**

Trial design



Conditions	*Salmonella* bath	Alphagos bath	Alphagos spraying
Negative control	No	No	No
2	Yes	No	No
3	No	Yes	No
4	Yes	Yes	No
5	Yes	Yes	Yes
6	No	Yes	Yes

2 chicken carcasses per condition

Salmonella enterica bath:1E5 CFU/ml, 20min at RT
Alphagos bath:1E7 PFU/ml, 20min at RT

25g of meat sampled before/after the trial for qPCR and CFU counting

Results

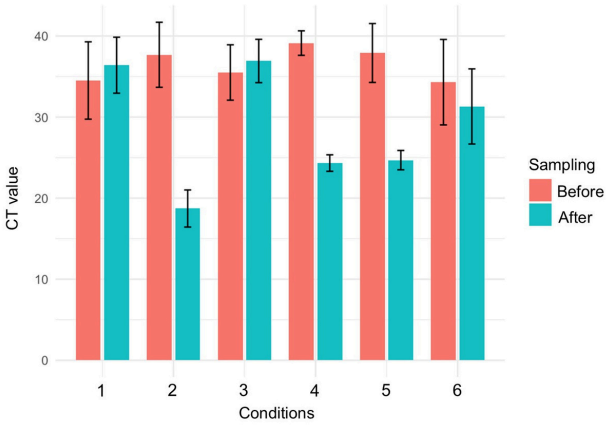


Figure 1 - Mean CT values obtained from qPCR analysis for six different conditions, measured both before (in red) and after (in green) the carcass processing. The qPCR was performed using the FoodProof detection kit targeting *Salmonella enterica.* Each bar represents the mean CT value from two biological replicates, with error bars indicating the standard deviation. A CT value higher than 30 is considered negative. Condition 1 represents the negative control and condition 2 the positive control. Conditions 4 and 5 have higher CT values compared to the positive control, indicating that the ***Salmonella enterica* load was reduced when carcasses were treated with Alphagos**. The difference in CT values between conditions 4/5 and condition 2 corresponds to ~64 times less target DNA on carcasses. **Alphagos** spraying at the end of the process did not affect the *Salmonella enterica* load, which indicates that this extra step is not needed for optimal *Salmonella enterica* reduction.

Key takeaways

- **Alphagos** reduces the load of *Salmonella enterica* in carcasses by 64x.
- **Alphagos** is stable in 1 ppm chlorinated water and **Alphagos** phages were still infecting after freezing.
- This study demonstrates the potential of phage cocktail as an **effective strategy against *Salmonella enterica* in poultry slaughterhouses**.
- Combining **Alphagos** in broilers and in carcass in the real conditions could lead to ***Salmonella*-free chicken products**.

* These authors contributed equally to this work; [1] Phagos SAS, Paris, France; [2] PT Vaksindo Satwa Nusantara, Indonesia

K1 Capsule-dependent phage-driven evolution in *Escherichia coli* leading to phage resistance and biofilm production

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* These authors equally supervised the work.
This research was financially supported by Wallonia in the framework of the call for projects organised by BioWin competitiveness cluster.

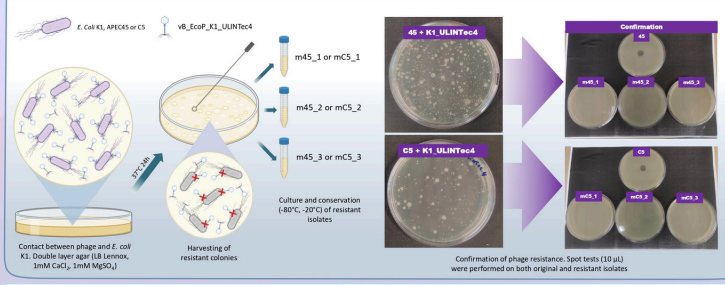
Introduction

Escherichia coli K1 is involved in several types of human infections, including meningitis, urinary tract infections and bloodstream infections. These strains can also be found in animals and particularly in poultry where they are responsible for colibacillosis. The understanding of the bacterial resistance mechanisms to phages has implications for the development of phage-based therapies. The objective of this study was to investigate the resistance of human and avian *E. coli* K1 isolates to K1_ULINTec4, a K1-dependent bacteriophage.

Material and methods

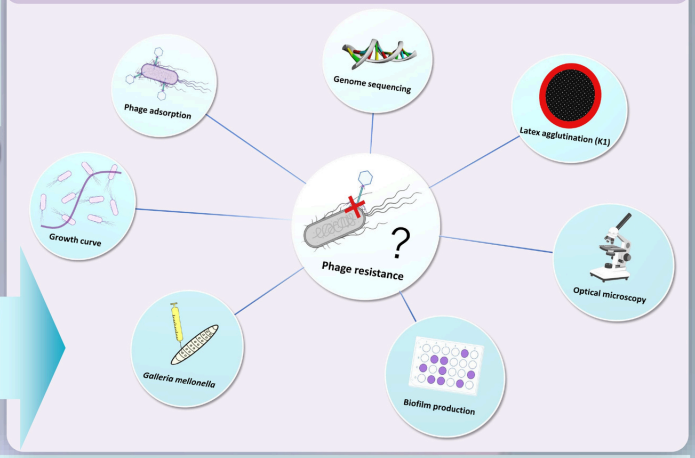
Resistant bacterial colonies were isolated from an avian pathogenic *E. coli* strain APEC 45 and the human strain C5, both previously exposed to K1_ULINTec4. After confirming their resistance, long read sequencing was performed, and several parameters were evaluated, such as growth capacity, phage adsorption, phenotypic impact at capsular level, biofilm production and virulence in the in vivo *Galleria mellonella* model.

Isolation of phage resistant colonies



Confirmation of phage resistance. Spot tests (10 µL) were performed on both original and resistant isolates

Characterization of phage resistant isolates



Results

One of the resistant isolates (mC5_2) exhibited a significantly slower growth rate in presence or absence of phage, suggesting the presence of a resistance mechanism altering its fitness (Figure 1). All resistant isolates showed decreased phage adsorption compared to the control (Figure 2). Comparative genomic analysis revealed insertion sequences in capsular genes (Figure 3). In addition, antigenic tests targeting the K1 capsule showed a very low positive reaction compared to the control (Figure 4). Nevertheless, microscopic images of resistant strains revealed the presence of capsules with a clustered organization (Figure 5) and biofilm assessment showed increased biofilm production compared to the original strains (Figure 6 and 7). In the Galleria model, larvae infected with phage-resistant strains showed better survival rates than larvae infected with phage-sensitive strains (Figure 8).

Growth curves

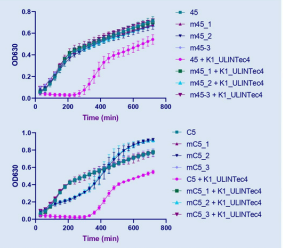


Figure 1. Growth curves of (up) APEC45 (45), (down) C5 and phage resistant isolates with or without addition of phage K1-ULINTec4. One resistant isolate (mC5_2) showed a slower growth rate.

Phage adsorption

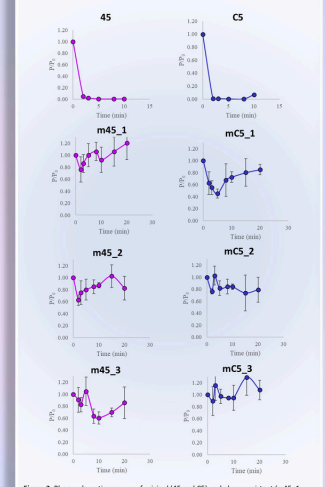


Figure 2. Phage adsorption curves of original (45 and C5) and phage resistant (m45_1, m45_2, m45_3, mC5_1, mC5_2 and mC5_3) isolates. P: number of free phages, P0: initial phage concentration.

Bacterial genome analysis

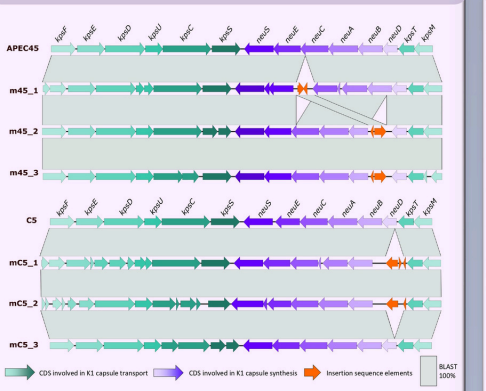


Figure 3. Capsular genes comparison of APEC 45, C5 and resistant isolates. Long read sequence assembly was performed using the BV-BRC platform with Canu 1.7.1 and error correction by Racon v1.4.13. Assembled genomes were then annotated with RASTtk and aligned with Mauve v2.4.0 to compare differences. EasyFig v2.2.5 was used to compare capsular genomic regions.

Impact on capsular production

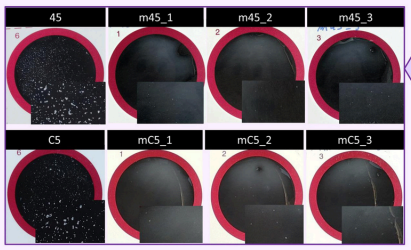
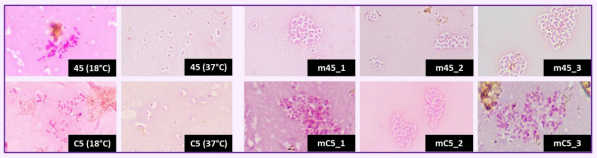


Figure 4. Pictures of the latex agglutination antigenic test (Wellcogen™ Neisseria meningitidis B/E. coli K1 Rapid Latex Agglutination Test) revealing the presence of K1 capsular antigens. A positive result produces small white agglutinations. Resistant isolates showed very weakly positive results .

Figure 5. Microscopic images (x100) of original (45 and C5) and phage resistant (m45_1, m45_2, m45_3, mC5_1, mC5_2 and mC5_3) isolates. Capsules are visible with negative Maneval staining (white halos around the bacteria). APEC45 and C5 cultures growned at 18°C were used as negative control since capsular production is inhibited at this temperature.



Biofilm production

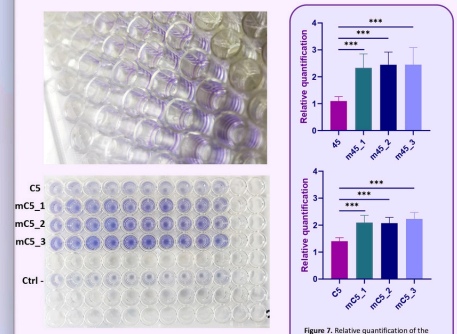


Figure 6. Pictures of 96-wells plates biofilm production assessment. (up) Surface biofilm formation staining after 24h incubation at 37°C and crystal violet suspensions (down) showing visual difference between original and phage resistant isolates.

Figure 7. Relative quantification of the biofilm production measured at 580nm of (up) APEC45 and phage resistant m45_1, m45_2, m45_3 and (down) C5, mC5_1, mC5_2, mC5_3.

Virulence in *Galleria mellonella*

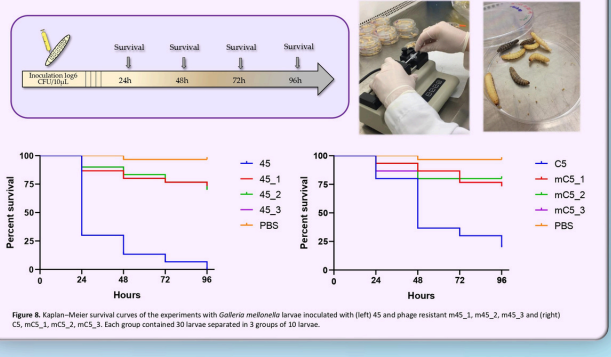


Figure 8. Kaplan–Meier survival curves of the experiments with *Galleria mellonella* larvae inoculated with (left) 45 and phage resistant m45_1, m45_2, m45_3 and (right) C5, mC5_1, mC5_2, mC5_3. Each group contained 30 larvae separated in 3 groups of 10 larvae.

Conclusion and perspectives

In conclusion, a phage resistance mechanism was detected at the genomic level, have an impact on capsular expression and was able to decrease the virulence of *E. coli* K1 in-vivo. Nevertheless, it is worth noticing the presence of capsules in the microscopic images and the increase in biofilm production in resistant isolates.