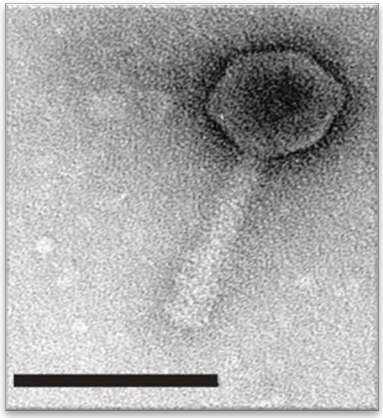
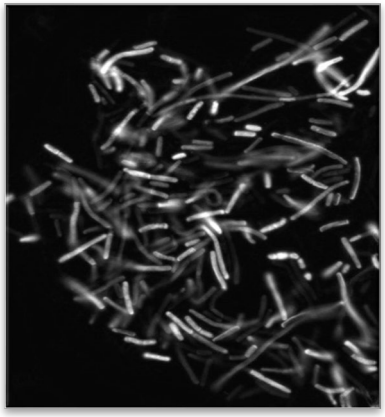
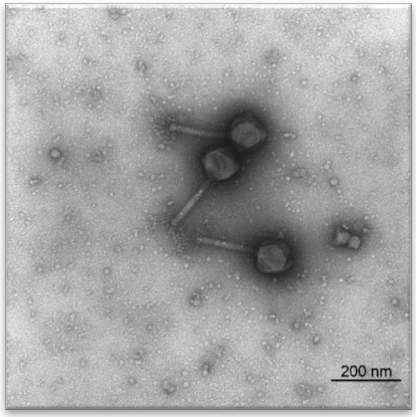
PTC PHAGE TECHNOLOGY CENTER







A bacteriophage product against *Staphylococcus aureus* to treat bovine mastitis

Hansjörg Lehnherr

IABS webinar:
Avoiding Antimicrobial Resistance: Veterinary use of Phages for Prevention, Therapy and Control of Bacterial Infections.

November 19-20, 2024; Online/ Zoom

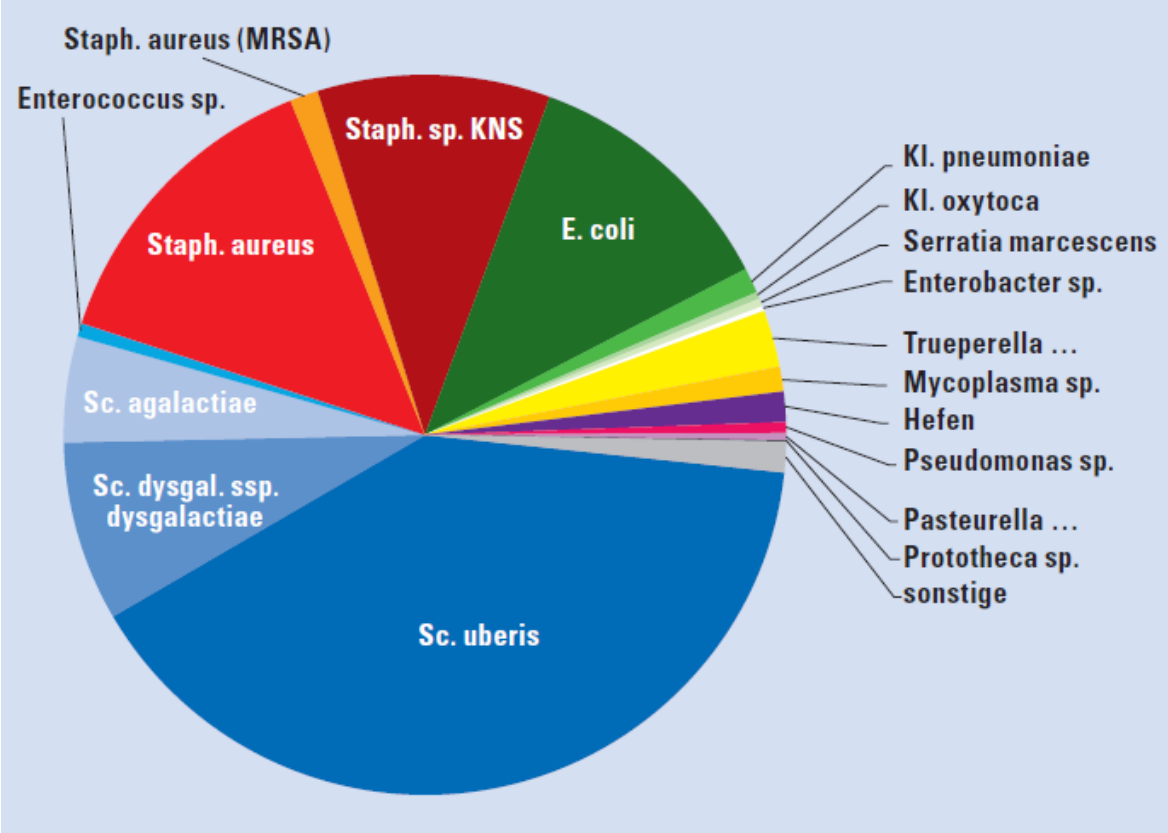
Bovine mastitis

Bovine mastitis is a polymicrobial disease manifested by an inflammation of the udder of a cow

- animal welfare
- significant cost factor for the dairy industry

J. Y. Nale and N. R. McEwan, "Bacteriophage Therapy to Control Bovine Mastitis: A Review," Antibiotics, vol. 12, no. 8, pp. 1–21, 2023

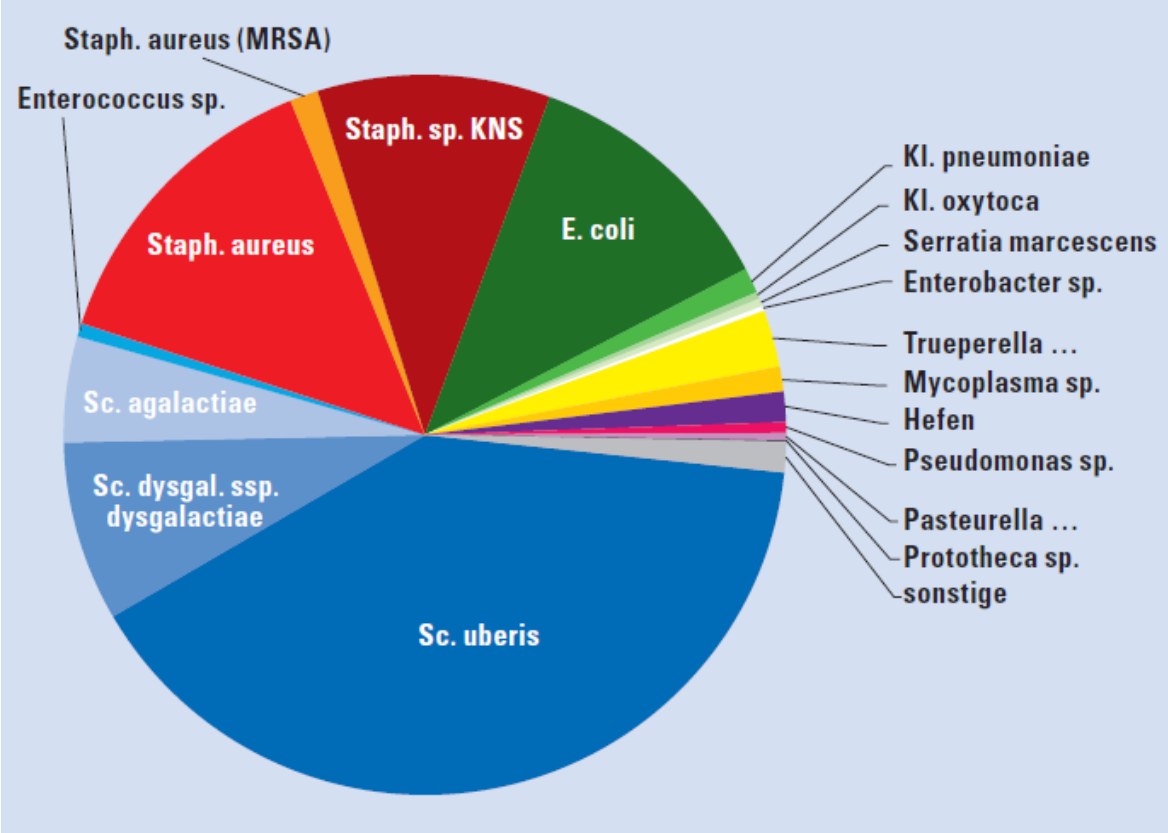
Causative agents



Annual Report TSK Saxony, 2021

- *Staphylococcus aureus*
- *Streptococcus uberis*

Causative agents



Annual Report TSK Saxony, 2021

- *Staphylococcus aureus*
- *Streptococcus uberis*

Somatic cell count (SCC)

The number of immune cells (white blood cells) per milliliter of milk.

Healthy cow: 50.000 to 100.000 cells/mL

Threshold levels:

Switzerland: 350.000 cells/mL

EU: 400.000 cells/mL

USA: 750.000 cells/mL

Standard treatment: antibiotics

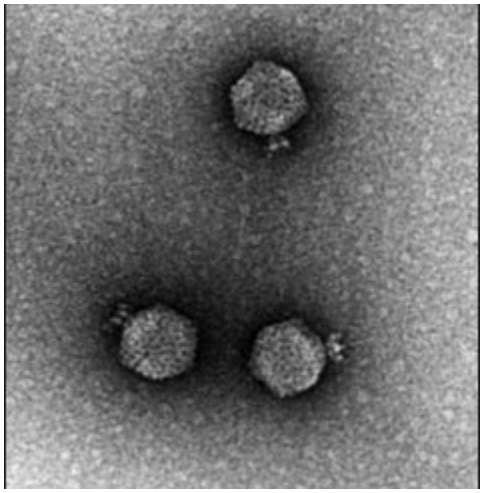
Current success rate of curing *Staphyoloccocus aureus* associated mastitis (during the lactation period) with antibiotics is lower than 50%!

J. Wilm, L. Svennesen, C. Kirkeby, and V. Krömker, "Treatment of clinically severe bovine mastitis – a scoping review," Front. Vet. Sci., vol. 11, no. January, pp. 1–11, 2024.

J. Y. Nale and N. R. McEwan, "Bacteriophage Therapy to Control Bovine Mastitis: A Review," Antibiotics, vol. 12, no. 8, pp. 1–21, 2023

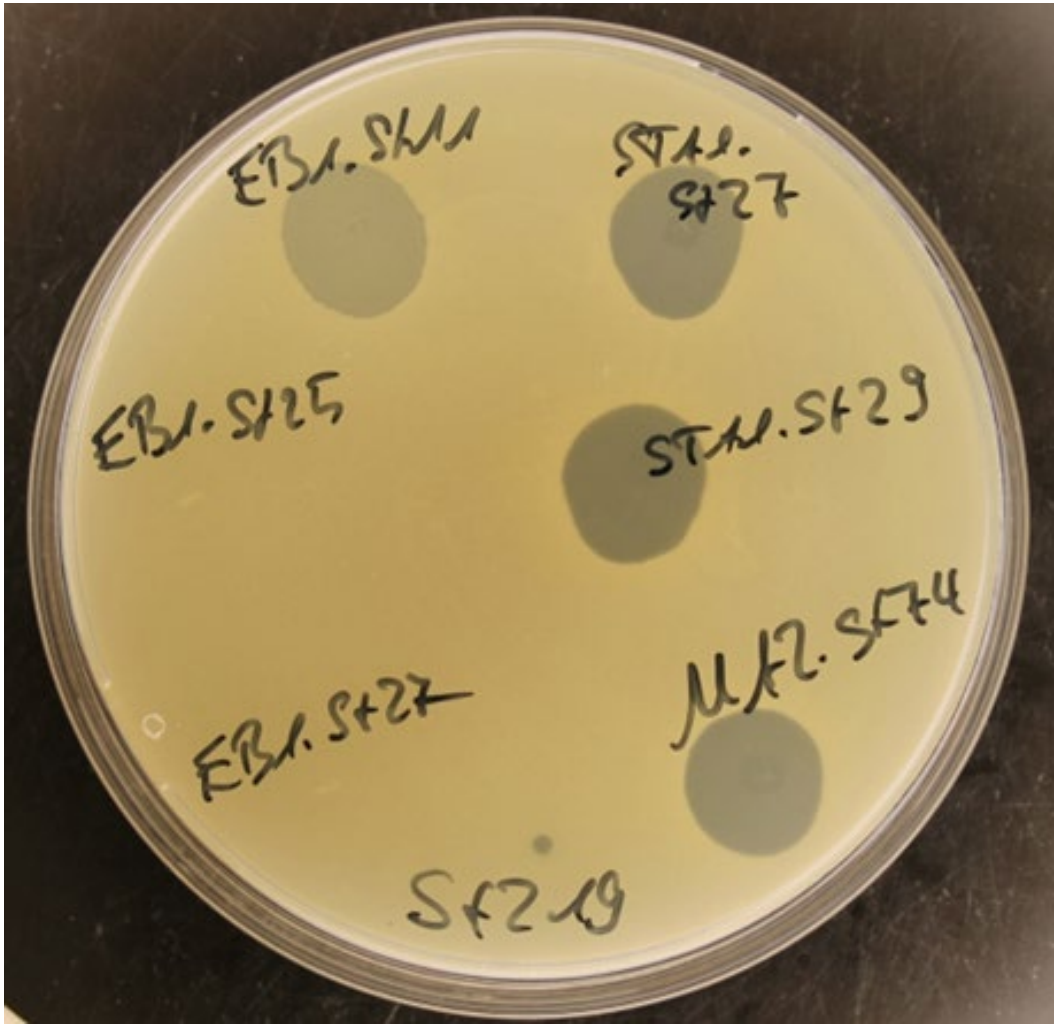
Bacteriophages against *Staph. aureus*

STA1
Myovirus
~140 kb
lytic



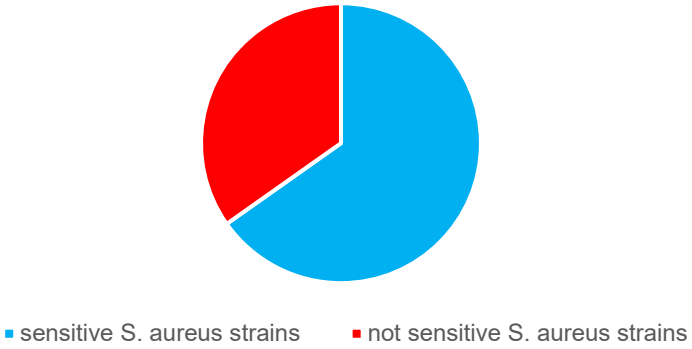
EB1
Podovirus
~17.5 kb
lytic

Coverage of mastitis-relevant isolates (spot test)

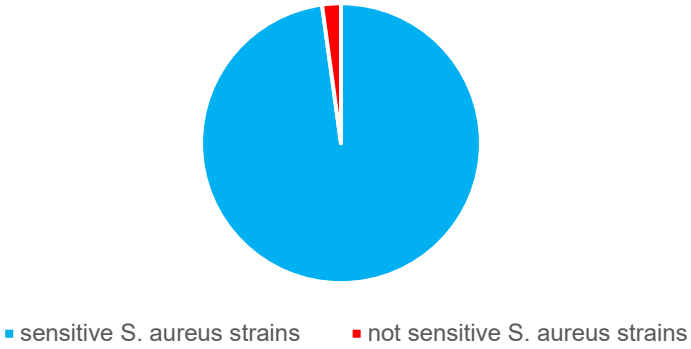


Product development

three component bacteriophage cocktail



five component bacteriophage cocktail



~ 97% coverage of relevant (46), mastitis-causing *Staphylococcus aureus* strains

Stability and activity in raw (unpasteurized) milk

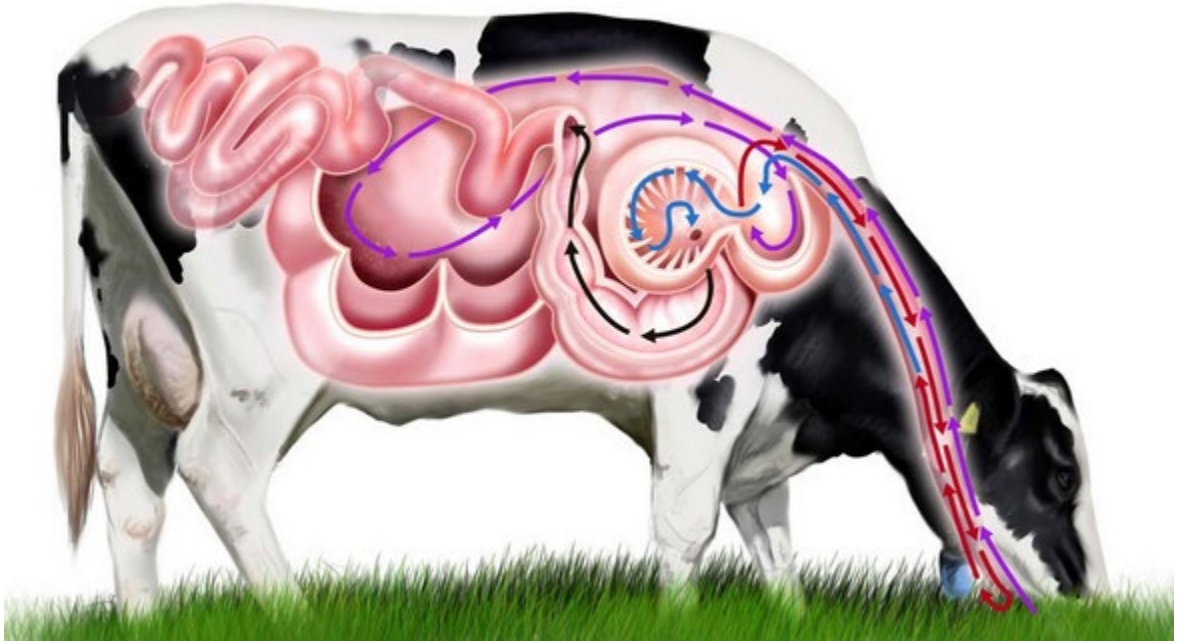
Composition of raw milk:

Water	87.2 %
Protein	3.5 %
Fat	3.7 %
Sugar	4.9 %
Antibodies	
Immune cells (SCC)	

In our hands the bacteriophages are:

stable	> 12 h
active	~ 1 log reduction of planktonic *Staphylococcus aureus* in 1 h

How to apply?



Oral application does not work.
External application, udder wash or udder dip does not work either.
Injection of a high dose into the teat channel!

- Veterinary medicinal product (VMP)

Diagnostic and treatment

Diagnostic:

- Detect first signs of mastitis (farmer)
- Identify *Staphylococcus aureus* as the cause (veterinarian)

Treatment:

- Milk the affected quarter(s)/gland(s) (farmer)
- Inject a high dose, e. g. 5 mL (1 x 10^9 PFU/mL), into the teat channel (veterinarian)
- Over a period of 12 hours the quarter is slowly filled with fresh raw milk (produced by the cow)
- This provides the bacteriophages with a 12 hour window to attack the *Staphylococcus aureus*
- Repeat six times over the course of three days

Field trial (n=2); diagnostic

Farm in the Bernese Emmental with 11 dairy cows

- Lili (red/brown) (40 L milk/day) with acute mastitis in all four quarters/glands
- Treatment with antibiotic (Kanamycin) failed
- Iasmin (black) (30 L milk/day) with acute mastitis in one quarter/gland
- Both cows tested positive for *Staphylococcus aureus*

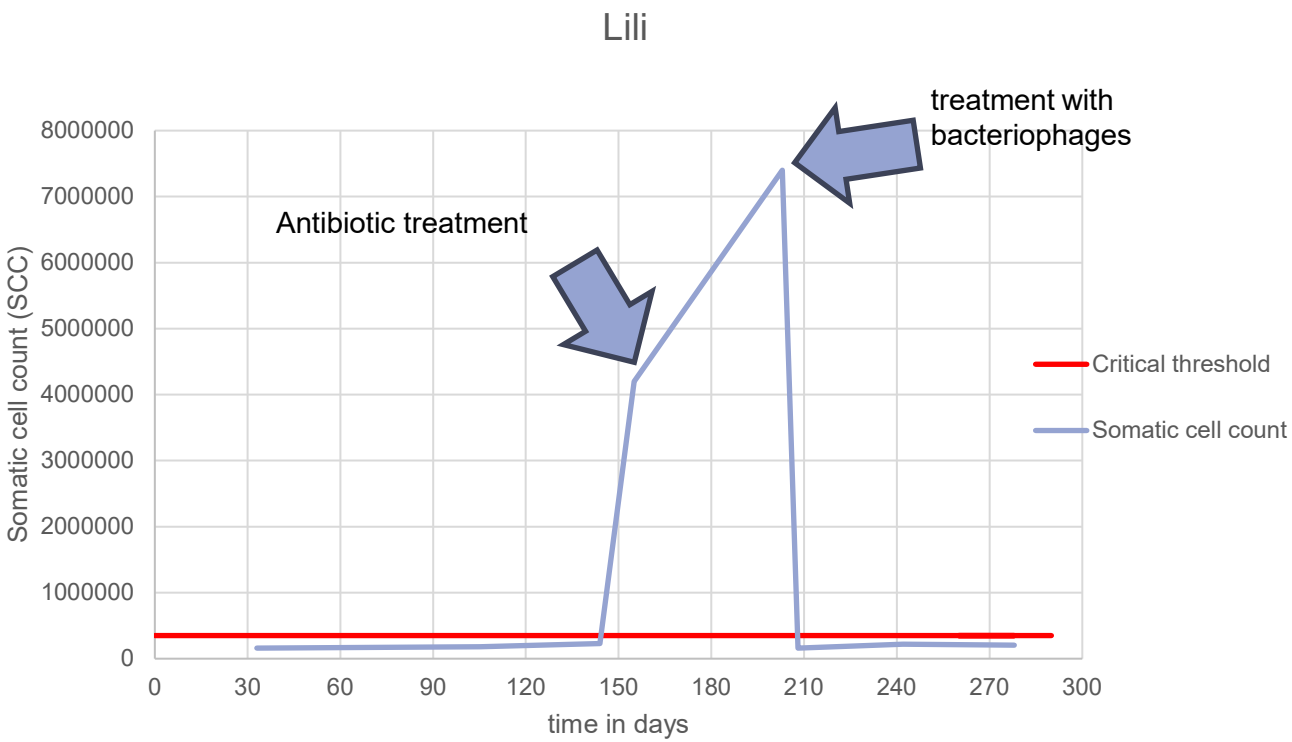
Field trial (n=2); treatment

Farm in the Bernese Emmental with 11 dairy cows

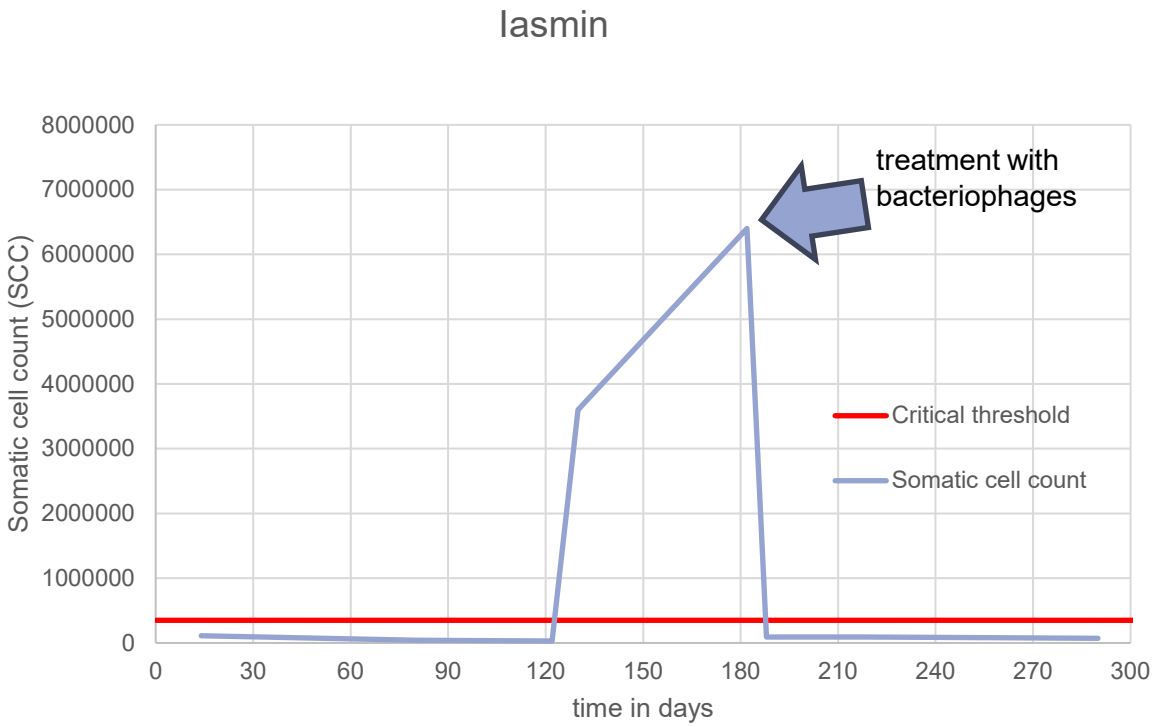
Treatment: Six injections over the course of three days:

- *Staphylococcus aureus* was no longer detectable in the milk
- The inflamation parameters disappeared rapidly
- No relapse occured

Lactation period Lili



Lactation period Iasmin



Publicly funded field trial, ongoing

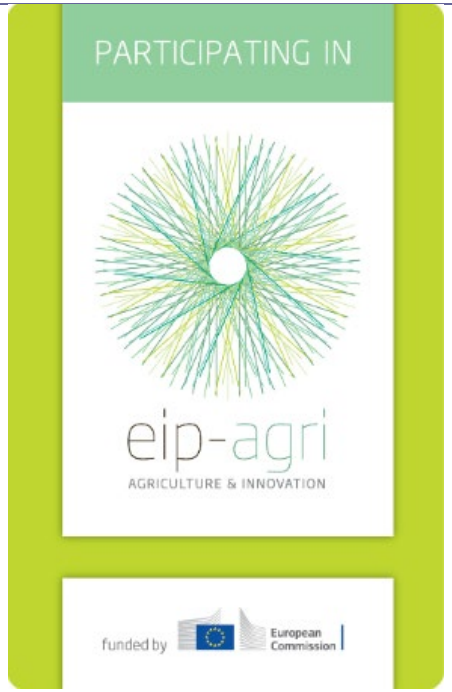
European Innovation Partnership

„Agricultural productivity and sustainability"

EIP-Agri

Lower Saxony

Germany



Application of bacteriophages in the therapy of *Staphylococcus aureus* mastitis"

University of Copenhagen:

Prof. Volker Krömker

University of Hannover:

Prof. Stefanie Leimbach

Dr. Nicole Wente

Dr. Anne Tellen

Dr. Janina Schmidt

PTC GmbH:

Dr. Hansjörg Lehnherr

Dr. Tatiana Lehnherr

Dr. Michael Fink

Dr. Lukas Lis

Dr. Andrea Kroj

Anika Faros

Dennis Otte

Mirko Knežević

Production of a bacteriophage cocktail

- Bacteriophage stock (MCB/WCB)
- Host strain (MCB/WCB)
- Fermentation (one step amplification)
- Purification (multistep filtration)
- Buffer exchange:
  - bacteriophage stability
  - food grade
  - freely miscible with raw milk
- Quality control:
  - Identity per PCR
  - Sterility
  - Purity (LPS etc.)
- Mixing of the components
- Sterile packaging

Product/capacity

Syringe filled with 4-6 mL of sterile product
Soft tip injector to protect the teat sphincter

PTC production capacity: 400 L per week
- enough to treat 13.000 quarters/glands per week

Treatment costs

Antibiotic treatment:

30 – 80 € (depending on the antibiotic)

Bacteriophage treatment:

25 - 30 € for 1 quarter
100 -120 € for all 4 quarters

Meat value of a dairy cow: 2500 – 3000 €
Average value of the milk produced by a dairy cow during a lactation period (year): 2800 – 3500 €

EMA

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

13 October 2023
EMA/CVMP/NTWP/32862/2022
Committee for Veterinary Medicinal Products (CVMP)

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

No veterinary medicinal product based on bacteriophages has yet been centrally authorized in the EU

Good Manufacturing Practice (GMP)

Current costs for GMP production (third party offers)
~ 250.000 € per liter per phage

Current costs for non-GMP production (PTC)
~ 120 € per liter per phage

treatment costs for a single cow (just the bacteriophage production):

GMP: 7500.00 €
non-GMP: 3.60 €

Meat value of a dairy cow: 2500 – 3000 €
Average value of the milk produced by a dairy cow during a lactation period (year): 2800 – 3500 €

IABS Webinar; Day 2: Wednesday, November 20, 2024

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

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

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

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

Thank you for your attention!

Phage Technology Center GmbH
Siemensstrasse 42
59199 Bönen
Germany
Tel: +49 (0) 2383 919 174; h.lehnherr@ptc-phage.com