PhageGuard

FOOD SAFETY POWERED BY NATURE

Phage usage in Poultry Processing

Content

1.Introduction

1.1 Micreos Food Safety

Micreos' vision is to ***Help As Many As Possible As Soon As Possible***. We pioneer in the field of targeted antibacterial technology and have developed cutting-edge proprietary products like PhageGuard for use on foods. Our mission is to:

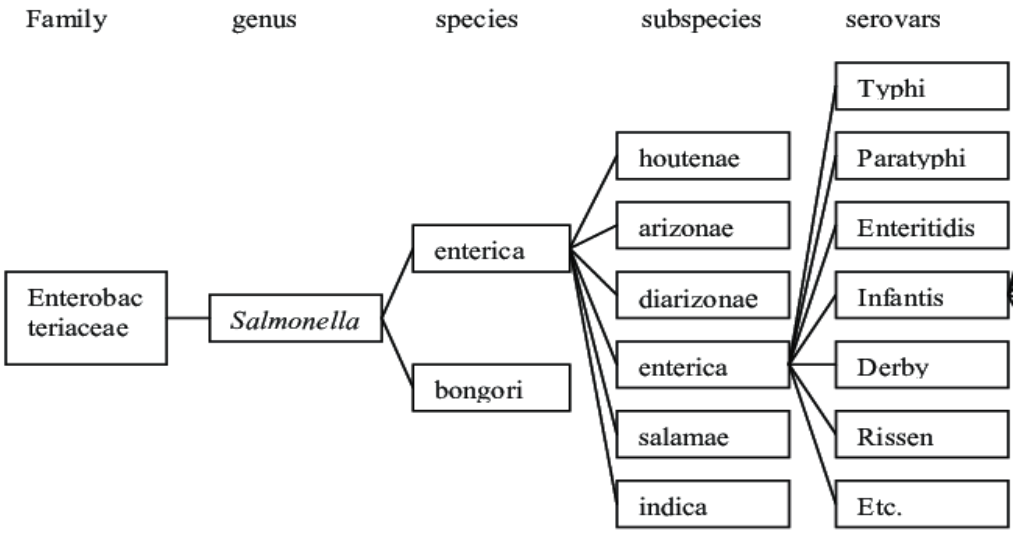
- Help safeguard human health. Especially children, the elderly and the immunocompromised.
- Improve the responsibility to provide a better quality of foods.
- Increase worker safety in food processing environments.

1.2 Bacteria and pathogenic bacteria

Nature carries many different types of bacteria. By far most of them are not harmful for human health or are even beneficial. Beneficial bacteria are used for instance during cheese making or in healthy drinks like Yakult. A few bacterial families do have members which can be detrimental to human health. For instance, certain subspecies of Salmonella, Listeria and E. coli. These are called "pathogens" and need special attention when food is produced.
In the Poultry industry special attention is given to Salmonella as these bacteria are often already present in the chicken, before it is processed into food.

1.2.1 Pathogenic bacteria species from genus *Salmonella*

The genus salmonella,



The *Salmonella* genus is a member of the family Enterobacteriaceae and contains two species, *Salmonella enterica* and *Salmonella bongori*, each consisting

of multiple serotypes. Non-typhoidal *Salmonella enterica* subspecies *enterica* is further divided into 2600 serovars (also called serotypes) and is known to cause human and animal gastroenteritis infection called Salmonellosis. Route of Salmonellosis infection in humans is a result of consumption of various type of uncooked meat and meat products.

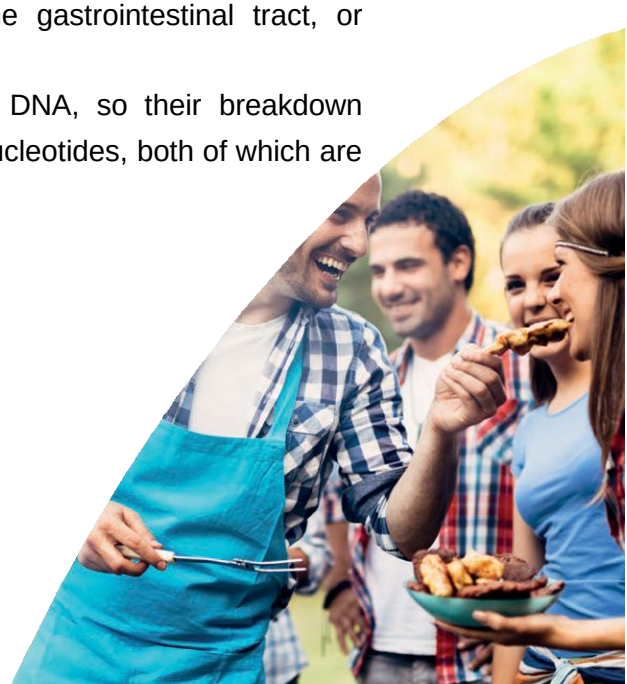
1.3 Bacteriophages

Bacteriophages ("**phages**") are the most abundant micro-organisms in the biosphere. Although many million times smaller than humans, their total weight exceeds the weight of the humans on our planet! They are the natural enemies of bacteria and are naturally present in very large numbers in water and foods of various origins. Phages are harmless to humans, animals, and plants. Humans are routinely exposed to phages at high levels through food and water without adverse effects. Phages use bacteria for their multiplication. Via this mechanism, phages contribute to environmental homeostasis, the situation wherein none of the bacterial species in a biosphere becomes dominant. Every subspecies of bacteria is thought to be the host for at least one phage. Several phages exist that can recognize and lyse (kill) a number of different bacterial strains within one genus; these have a 'broad spectrum' or a wide host range.

Thus, bacteriophages can be regarded as natural enemies of bacteria, and are therefore logical candidates for targeted control of food borne bacterial pathogens like *Salmonella*.

Unique attributes of bacteriophages include:

- Phages kill only bacterial target cells (no impact on plant or animal cells).
- They do not cross genus boundaries; therefore, our Listeria and *Salmonella* phages will not affect other desired bacteria in foods (e.g., starter cultures for cheese and sausages), and commensals in the gastrointestinal tract, or accompanying bacterial flora in the environment.
- Phages are composed entirely of proteins and DNA, so their breakdown products consist exclusively of amino acids and nucleotides, both of which are present in abundance in food products.



Regulatory

PhageGuard S is for both USDA and FDA GRAS (GRN000468). Processing aid approvals for USDA appear in 7120.10. It is further approved as a processing aid in Canada, Australia, Israel, and others. It is organic certified (OMRI USA and SKAL EU), Halal and Kosher.

Safety

With respect to their potential application for the biocontrol of undesired pathogens in foods, feeds, and related environments, it should be considered that phages are the most abundant micro-organisms in our environment and are present in significant numbers in water and foods of various origins, in particular fermented foods (reviewed by Sulakvelidze and Barrow, 2005). On fresh and processed dairy and meat products, more than 10^8 viable phages per gram are often present (Kennedy and Bitton, 1987). It is a fact that phages are routinely consumed with our food in high numbers. Moreover, phages are also normal commensals of humans and animals, and are especially abundant in the gastrointestinal tract (Furuse, 1987; Breitbart, 2003).
In conclusion, bacteriophages are known to be harmless for all other organisms and are species-specific.

Phages have been successfully used for over 10 years in the food industry.

Over the past 10 years, many US companies have used *Listeria* phages to eliminate *listeria* and reduce risk in in RTE meats, cold smoked fish and soft cheese applications as well as an environmental tool to take out biofilms.

1.4 Micreos' bacteriophage product against Salmonella: PhageGuard S

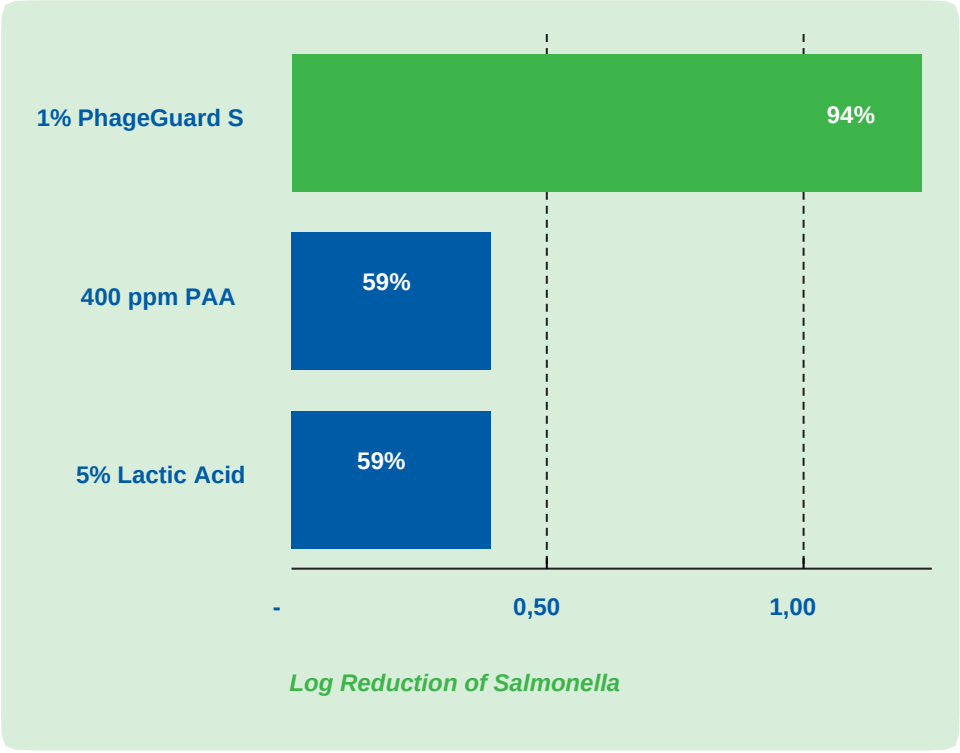
PhageGuard S is a water-based phage solution which contains *Salmonella*-specific bacteriophages. It is approved for commercial use by both FDA and USDA as a processing aid. PhageGuard S infects all *Salmonella enterica* serovar (also known as serotypes).

- Proven effective against USDA list of "top 20 prevalent" *Salmonella* serovars plus more
- Effective against all antibiotic resistant strains: phages use a different attack mechanism as they "don't care" about presence of antibiotic resistance genes in the strains
- Thus far no incidences of phage-resistant isolates have been identified

Non-Clinical Non-human 2012			
Rank	**Serotype**	**Reported**	**Percent**
1	Kentucky	1050	14.9
2	Enteritidis	932	13.2
3	Heidelberg	837	11.9
4	Senftenberg	643	9.1
5	Typhimurium	423	6.0
6	Mbandaka	316	4.5
7	Montevideo	184	2.6
8	Thompson	153	2.2
9	Muenster	150	2.1
10	Braenderup	140	2.0
11	Schwarzengrund	135	1.9
12	Newport	131	1.9
13	Anatum	122	1.7
14	Hadar	110	1.6
15	Infantis	110	1.6
16	Liverpool	88	1.3
17	Cerro	87	1.2
18	Agona	80	1.1
19	Dublin	61	0.9
20	Muenchen	61	0.9
		5813	**82.5**
	All other serotype	1165	16.5
	Rough, mucoid, and/or nonmotile isolates	6	0.1
	Unknown	58	0.8
	Subtotal	**1229**	**17.4**
		7042	**100**

1.1 Features of PhageGuard S compared to chemicals used in industry

- Organic and natural antimicrobial intervention which kills all *Salmonella*
- Tasteless and odorless, no impact on the organoleptic properties of foods
- Zero exposure risks regarding workers safety
- Cannot affect or influence “starter cultures” or the “natural microbial flora” on raw materials and finished foods
- PhageGuard S applied as an added hurdle on carcass, pre-grinding, or pre-packaging, reduces *Salmonella* up to 2-logs (99%)
- PhageGuard S outpaces common chemicals such as PAA and CPC (cetylpyridinium chloride) or organic acids on meats



(1) *Effect of ultraviolet light, organic acids, and bacteriophage on Salmonella populations in ground* Yeh Y, de Moura FH, Van Den Broek, de Mello AS (January, 2018) .

2. Scientific efficacy data on poultry products

2.1 Key parameters supporting phage efficacy:

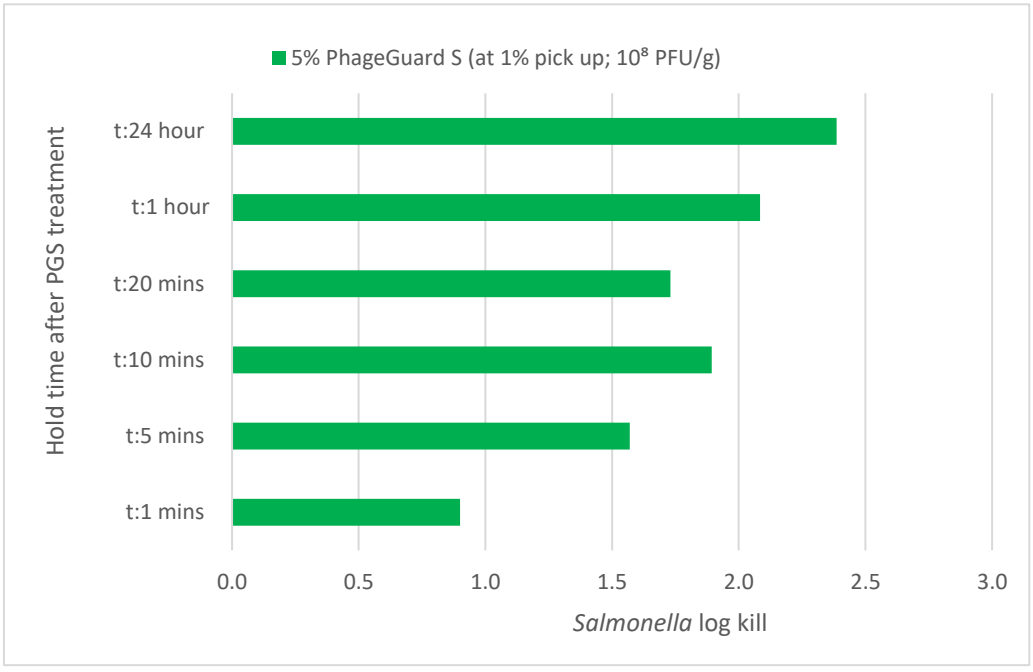
1. **Dwell time:** Hold time after phage treatment before next processing step.
2. **Pick up:** Volume of phage solution applied in order to have good coverage on product surface thereby allowing phage diffusion. It is commonly expressed as percentage liquid volume per weight (% v/w).
3. **Target phage concentration:** Optimal phage concentration on foods recommended by researchers based on type of product and surface. It is expressed as Plaque forming units per gram (PFU/g).

Reference scale for quantitating phage efficacy:

Log kill	1-log	2-logs	3-logs	4-logs	5-logs
Bacterial reduction	90 %	99 %	99.9 %	99.99 %	99.999 %

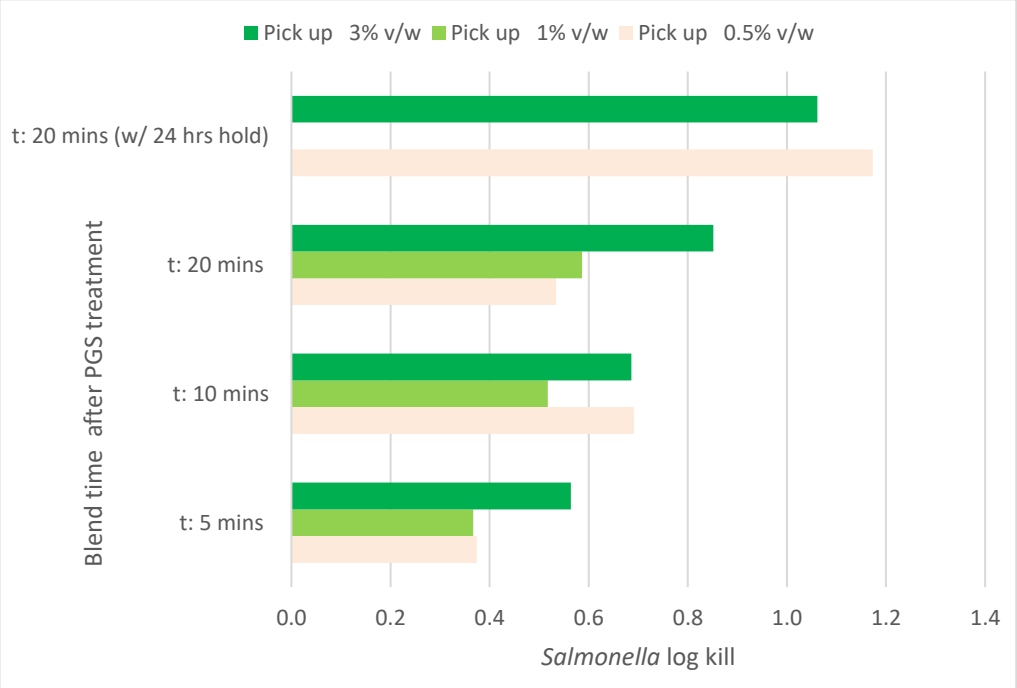
2.2 Internal research studies

Effect of dwell time after PhageGuard S (PGS) treatment on individual chicken breast fillet on a line or packages



- 5 minutes into treating with phages, PGS reduces 96.14% (1.6 log kill) of *Salmonella* on chicken fillet. Increasing hold time (~18 h) results in higher *Salmonella* kill up to 99.55% (2.4 log kill).
- With the possibility of only short dwell times before the next processing step, use of higher PGS concentration results in faster and larger *Salmonella* kill.

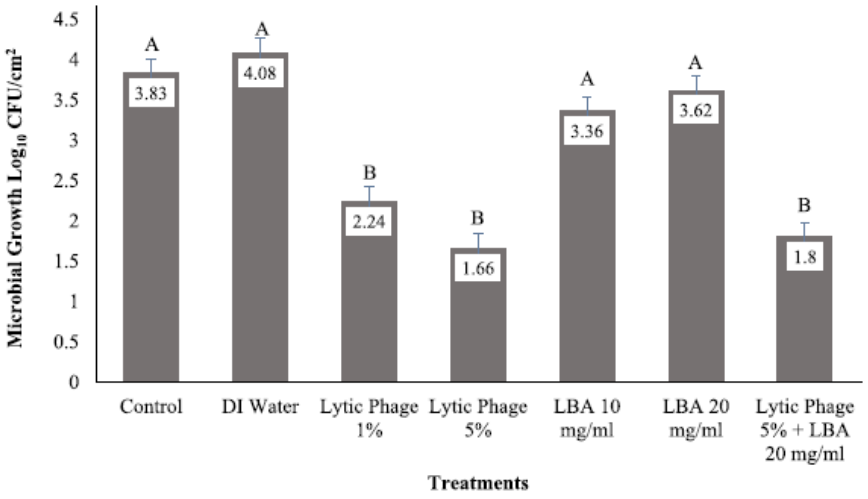
Effect of pick up and blend time for good distribution of PGS on pieces of chicken breast fillets in a tumbler



- Note: pick up level is inversely proportional to phage concentration.
- On irregular surfaces (parts, trims) applying higher pick up levels helps give good coverage.
- In case pick up level is a constraint on a product, longer hold “dwell” time (~18 h) helps to get considerable kill irrespective of pick up level.
- Longer blend times increase homogeneous phage diffusion on surfaces and thereby enhances bacterial kill (recommend minimum 10 minutes).
- In case of short blend times for operational reasons, higher pick up levels (recommend minimum 1% v/w) results in substantial bacterial reductions.

2.3 Published research and 3rd party lab validation study chicken parts

Salmonella Typhimurium DT 104 response to Lytic bacteriophage and Lactobionic acid (LBA) on raw chicken breast. Walker *et al.* (June, 2021)



A, B, C Groups lacking common superscript letters are different ($P<0.05$).

Fig. 2. Effect of different antimicrobial treatments on *Salmonella* Typhimurium counts on chicken breast across all sampling hours. Values are means of CFU/cm² with different treatments.

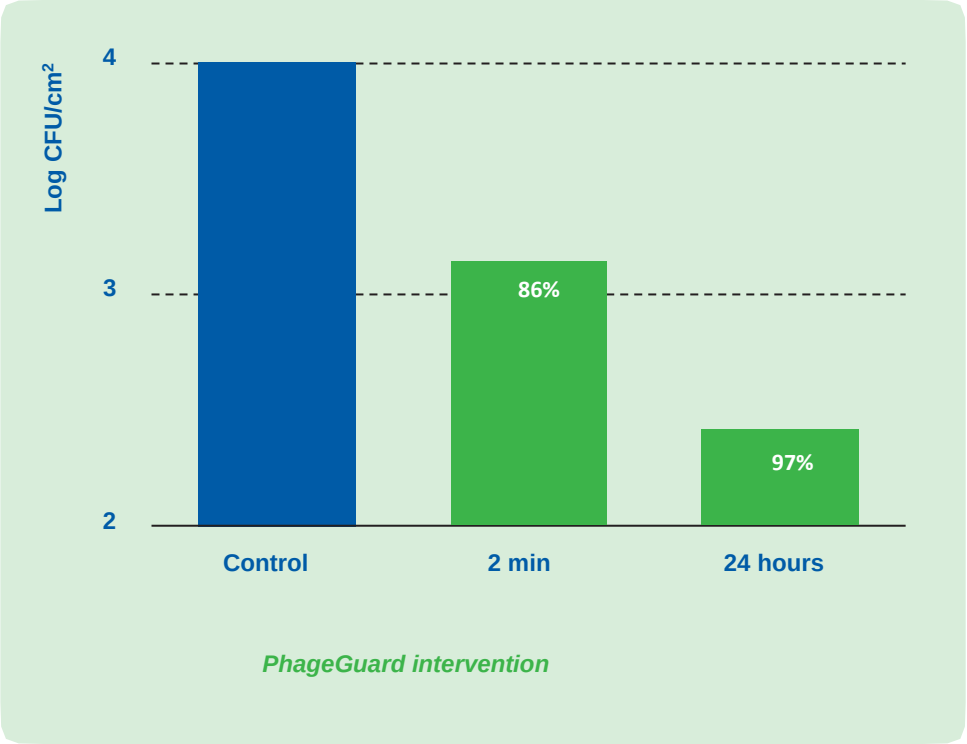
- Individual treatment with 1% bacteriophage solution (PGS) on chicken breast resulted in a significant *Salmonella* reduction (1.6 logs or 97.48%). Applying higher bacteriophage concentration (5% PGS) resulted in even higher *Salmonella* kill (2.2 logs or 99.36 %).
- Phages even at 1% concentration outcompeted highest concentration LBA treatment in terms of efficacy against *Salmonella*.
- There was no added benefit in combining LBA with phages as most of the *Salmonella* kill was because of PGS regardless in combination or individually.

(3) *Salmonella Typhimurium DT 104 response to Lytic bacteriophage and Lactobionic acid on raw chicken breast. Nicole Walker, Sherita Li, Hannah Strauss, Siroj Pokharel (June, 2021)*

Certified Labs, USA (2015) – PGS on chicken thighs (skin on, bone-in)

Studies have confirmed that by applying PhageGuard S on fresh meat pre- grinding or pre-packaging, *Salmonella* is reduced by up to 99%.

In a typical lab study performed by Certified Labs, skin-on, bone-in chicken thighs were contaminated with *Salmonella* and then treated with 1% PhageGuard S at 0.5% v/w pick up. Efficacy was within minutes and increased over time up to 24 hours after application.



2.4 Industrial trials

Multiple trials in various poultry plants at different geographical locations have shown that PhageGuard S can be easily applied and gives significant reduction in *Salmonella* positives on

- Poultry carcass
- Poultry parts
- Ground poultry product

PGS application on poultry carcass (South Africa)

In the trial, more than four million carcasses were treated with a commercial batch of bacteriophages over a period of four weeks. The carcasses were sprayed with 1% PhageGuard S solution and sampled before entering and after exiting the spin chiller.

Bacteriophages breakthrough

While Europe leads the charge for and the technology to achieve ABF poultry production, South Africa recently recorded an industry-first in the use of bacteriophages to reduce *Salmonella* loads in chicken-processing facilities.

A bacteriophage is a type of virus that infects and destroys bacteria. In fact, the word "bacteriophage" literally means "bacteria eater".

The beauty of bacteriophages is that they only kill their target organism, with no impact on other organisms or the background environment. They are also completely natural and occur abundantly in nature.

The role bacteriophages can play in food safety is a well-researched discipline, but up to now studies were confined to laboratories. That all changed in 2020 when the Centre for Food Safety at Stellenbosch University conducted a field study in an operating chicken abattoir to determine the efficacy of bacteriophages to destroy *Salmonella* on chicken carcasses. (*Salmonella* is particularly worrisome, given that it is becoming resistant to almost all antimicrobials.)

In the study, more than four million carcasses were treated with a commercial bacteriophages cocktail over a period of four weeks. The carcasses were sprayed and sampled before entering and after exiting the spin chiller.

Professor Gouws says the study proved that bacteriophages, combined with chlorine wash, are effective against multidrug-resistant *Salmonella*. "When applied correctly, bacteriophages can greatly improve food safety, with no adverse impact on abattoir workers, consumers or the environment," he says.

In commenting on the study, the GM of SAPA's Broiler Organisation, Izaak Breitenbach, said that the association supports the move away from antimicrobials. "Bacteriophages can greatly enhance food safety in South Africa, especially given our warm climate in which bacteria multiply quickly and easily," he says.

Photographs: Shutterstock.com

Professor Pieter Gouws of South Africa's Stellenbosch University concluded that the study proved that bacteriophages, combined with chlorine wash, proved effective against multidrug-resistant Salmonella. "*When applied correctly, bacteriophages can greatly improve food safety, with no adverse impact on abattoir workers, consumers or the environment*", he says.

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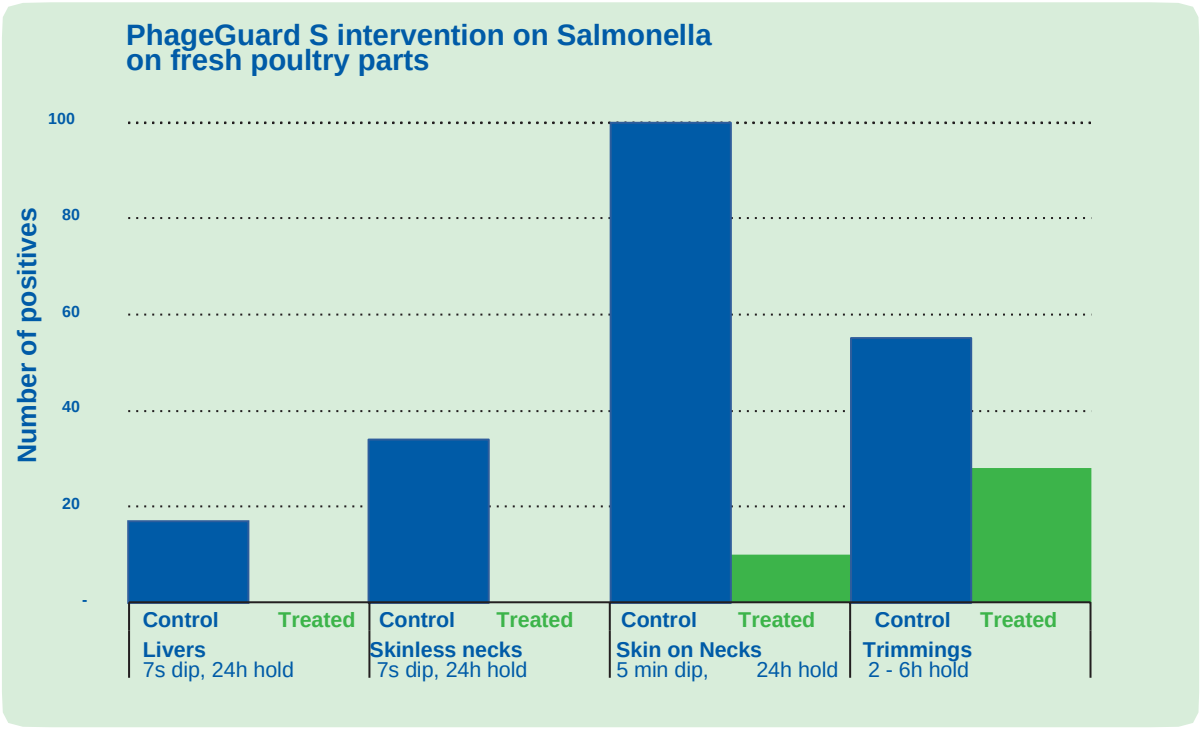
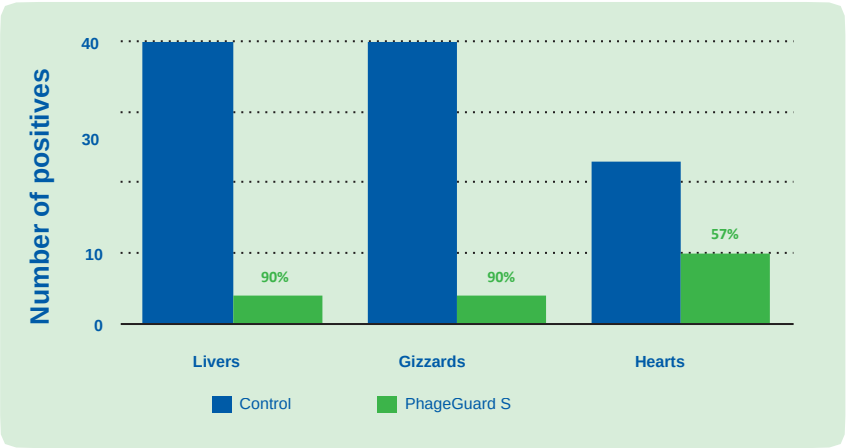
PGS application on poultry parts (United States of America)

On fresh poultry parts



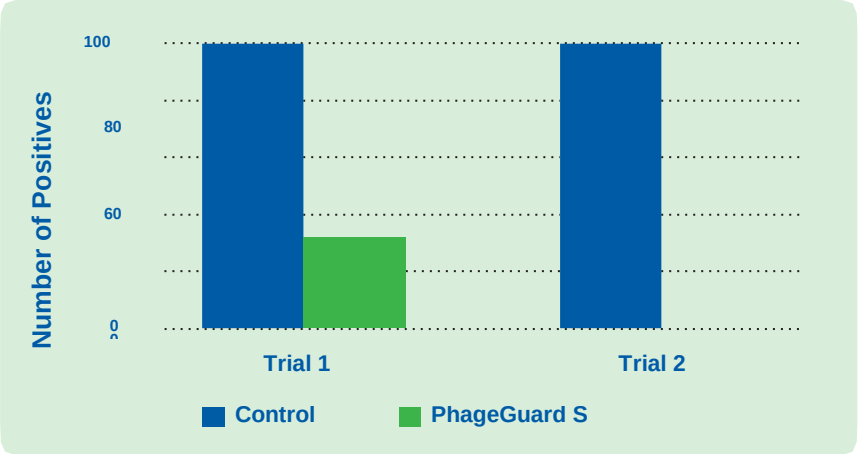
PhageGuard efficacy has been proven in multiple plant trials. The number of *Salmonella* positives reduced significantly. Number of positives for livers came down from 17% to zero; skinless necks from 34% to zero; skin on necks from 100% to 10%. For trimmings, the number of positives was reduced by approximately 50%.

Other chicken parts - livers, gizzards and hearts



Naturally contaminated Livers and Gizzards were dip treated with 1% PhageGuard S or tap water (control) at 4 ºC and subsequently crust frozen for 24 hours. The samples were rinsed and tested following the USDA method. Internal work performed at Micreos as well shows that dipping in 1% PGS solution for 15 minutes results in a 1-log (90%) reduction on livers and a 1 log reduction on hearts.

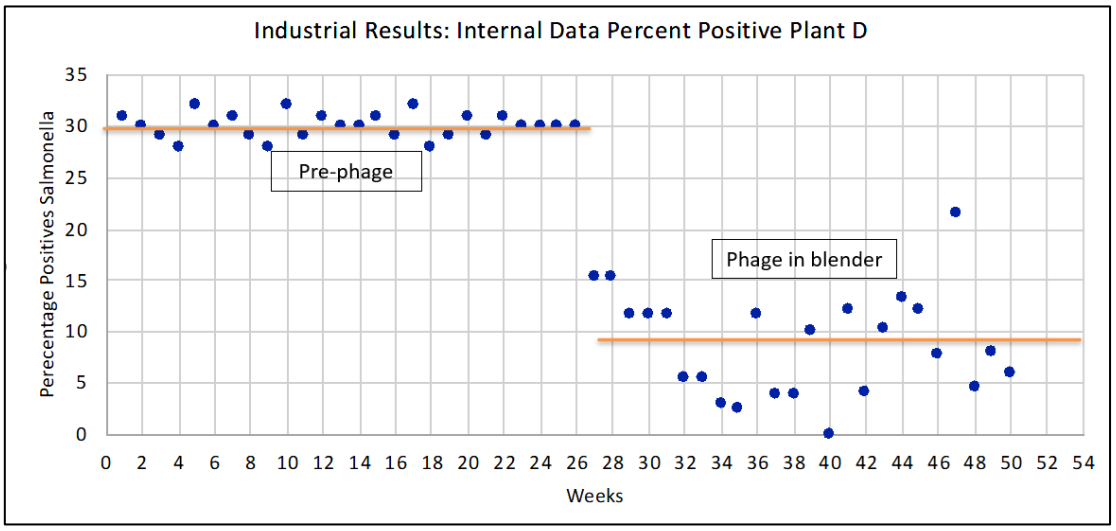
Turkey Backs & Wings



Turkey backs and wings were processed through a commercially available dip tank auger system using 4% PhageGuard S and then ground in a POSS separator. All chemical interventions had no effect while PhageGuard S reduced the number of positives up to 100%.

PGS application in ground poultry products (United States of America)

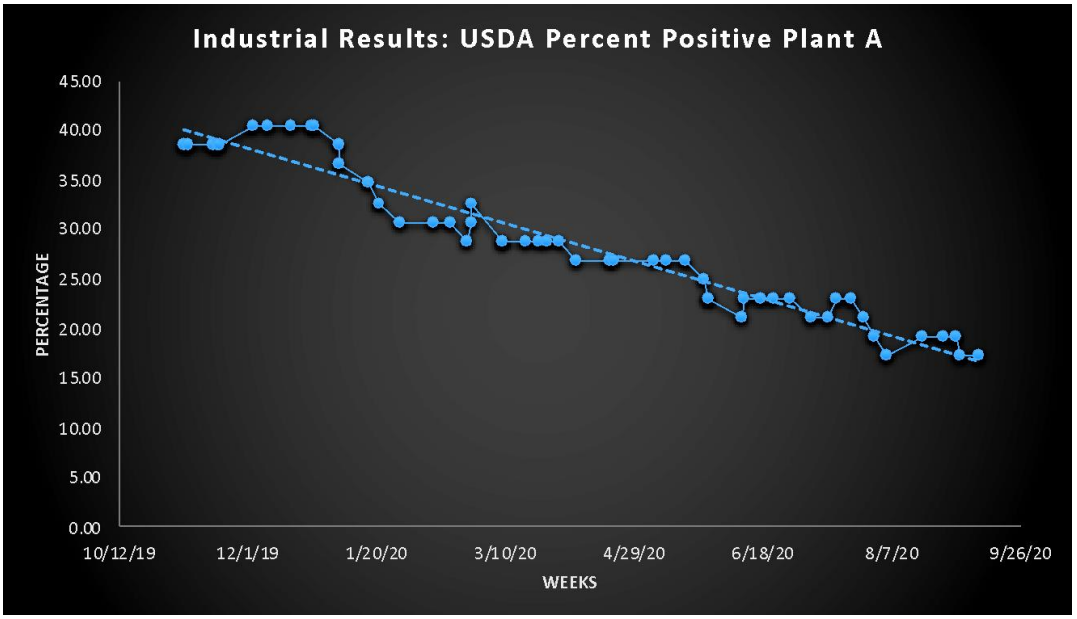
Customer 1 application point and dose - POST CO2 flush: 5% PGS dosage at 1% pick up.



Results:

Plant showed that *Salmonella* positive rate per week ranged from 30% +ve's on average before phage treatment and after phage treatment positives per week lowered to 9.6% +ve's on average over 28 weeks trial period. This shows that PhageGuard S when applied post CO_2 on ground in the blender step was able reduce the *Salmonella* prevalence by 68% in the final ground turkey product.

Customer 2 application point and dose - PRE CO2 flush: 5% PGS dosage at 1% pick up.



Results:

For another instance, a different customer plant was adding PhageGuard S to ground turkey at the blender step PRE CO_2 flush. USDA monitoring showed that *Salmonella* positives in samples collected at plant was approximately 38% before phage use. Over course of using phages for 50 weeks, *Salmonella* positives in samples collected by USDA decreased from 38% +ve's down to 17% +ve's. In this way, PhageGuard S was effective in reducing the *Salmonella* prevalence by 55% in final ground poultry product.

3. Delivery system data: Spray bar

Spraying phages

This section describes the effect of the spraying conditions on the phage stability and the parameters that need to be considered. Different nozzles and spraying conditions have varying effects on phages.

Goal

This section is intended to provide findings and establishing guidelines based on these findings, on how to spray phage solutions with a minimal loss of activity.

Effect of spraying on phage efficacy

Under specific circumstances, phages can be sensitive to spraying because of 2 main reasons.
1: Phages adsorb to the air water interface which can lead to inactivation. As a result, the surface per unit of volume should not be too high because the number of phages adsorbed will not be negligible in comparison to the total number of phages.
2: Very small droplets have a very high internal pressure due to their surface tension (Laplace pressure) which will lead to a very rapid dehydration of the droplets will lead to the inactivation of phages.
As a result, we advise not to decrease the droplet size too much typically not much lower than 150 µm because it will have consequences for the phage activity. In the information below several combinations of pressure, nozzle and flat spray or air atomizing have been tested to evaluate spraying.

Brief summary of past findings & results

- In general, the droplet size should not be too small because in that case the surface to volume ratio is too high, which leads to evaporation of water, and an excess absorption of the phages to the air water surface. As a rule, the size of the droplets should not be much lower than 150 µm Sauter mean diameter.
- We recommend the spraying of phage solutions by a flat spray nozzle and not by means of air atomisation. This has to do with the wider size distribution of the droplets caused by air atomisation in comparison to the flat spray nozzles.
- If the spray is completely homogeneous over the surface of the amount of liquid per cm^2 would be in the order of 13 µl. In general, we advise to use 25 $\mu l/cm^2$ which also makes 150-200 µm droplets very suitable to spray.
  If the distribution of the solution is not homogeneous over the surface because the droplet size is too large it is generally better to further dilute the phage solution rather than adjusting the droplet size.
- Our partner is a worldwide supplier: Spraying Systems Co. Otto Jouwsma is our primary contact, also for foreign clients.
- The most common type of spray system we use is regular, mechanical with pressure. We have used electrostatic spraying (Sono-tec) in the past, yet this is comparatively much more expensive (>€ 100k).
- We generally use between 1 to 2 bar of pressure. Above this phages deteriorate.

Past spray data

In the sections below several different experiments and calculations are made that provide background information on the relation between phage application and the spraying parameters.

Phage application efficiency

U In order for phage to work properly phage needs to be diluted to a certain extent for the phages to be mobile on the surface. The amount of liquid per cm^2 is recommended to be in the order of 15 to 30 microliters for the phage to work properly. Ideally the spraying should consist of droplets that have a size consistent with the amount of liquid that is needed per cm^2. In the graph below an estimate is given of the volume that needs to be sprayed per cm^2 if we consider that all droplets are neatly aligned side by side. In that case we find for a droplet size of 200 µm a volume of around 13 µl. To obtain a good distribution we can assume that 2 layers of droplets should suffice. In figure 2 a small part of the graph in figure 1 is shown. In this graph we can observe that in that case the droplet size should ideally be between 150 and 220 microns.

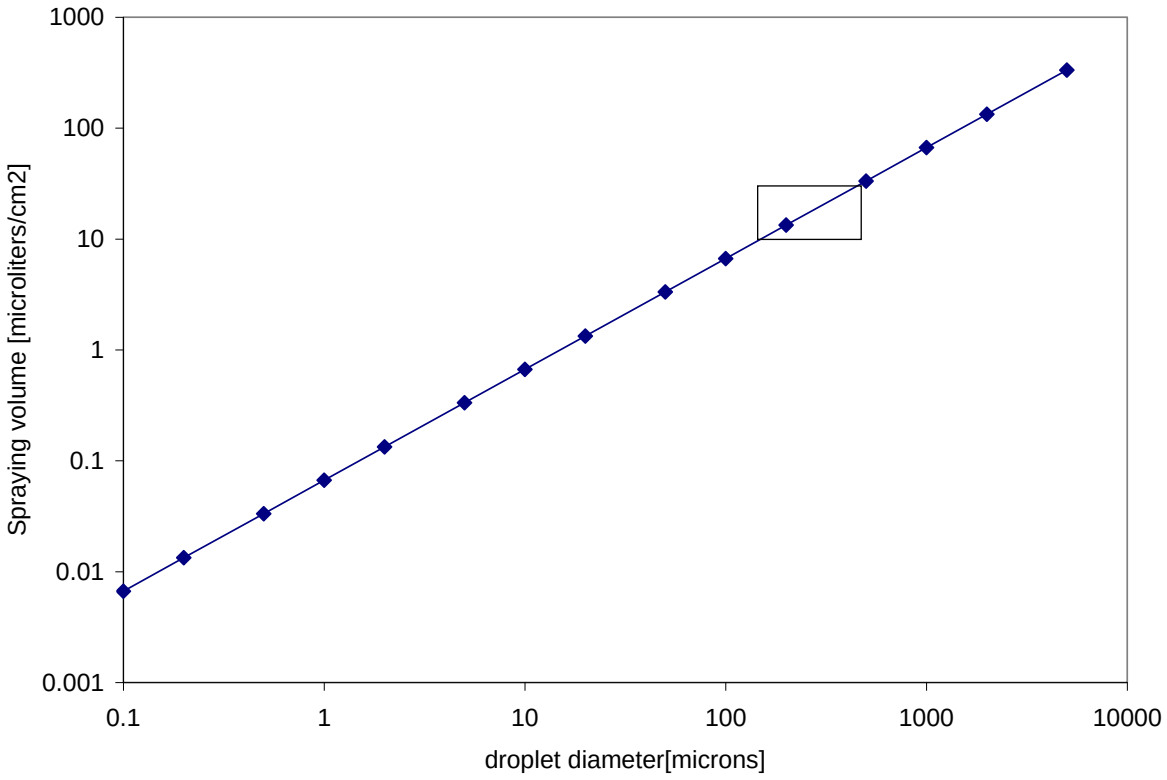


Figure 1: Relation between droplet diameter and surface load (ideal) in microliters/cm^2.

Phage tolerances for different nozzles

(Nozzle origin: Spraying systems Co.)

Nozzle code	Orifice diameter at 3bar	Spray pattern angle at 3 bar	Flow rate at 3 bar
(TPU)650050-SS	457,2 µm	65	0,19 lpm
(TPU)800050-SS	457,2 µm	80	0,19 lpm
(TPU)650067-SS	533 µm	65	0,25 lpm
(TPU)800067-SS	533 µm	80	0,25 lpm
(TPU)1501-SS	660 µm	15	0,37 lpm
(TPU) 6501-SS	660 µm	65	0,37 lpm
(TPU) 8001-SS	660 µm	80	0,37 lpm
(TG)SS0.7(cone nozzle)	760 µm		

Further information

In the appendix additional information is provided on the spraying characteristics of spraying performance distance for flat spray nozzles. Also, a table is provided that gives more information on the relation between nozzle type and capacity depending on the requirements.

Preferred settings for flat spray nozzles (rules of thumb)
1 – 2 bar of pressure, with a max of 3 bar pressure.
65^{o} spray fan (is 65^{o} at 3 bars).
15 cm distance to target surface area.
30% overlap from spray edge to spray edge.

From table, page A5. Industrial Spray Products, Catalog 70-M, Spraying Systems Co.

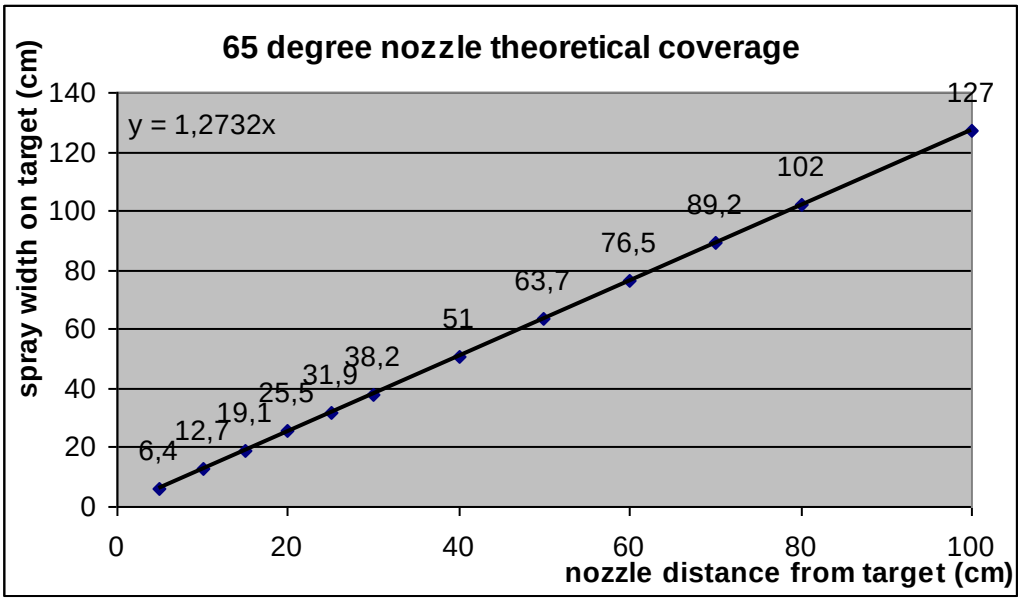
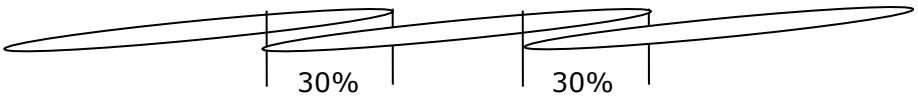


Figure A1: Relation between nozzle distance and spraying width

Example: 65^{o} nozzle at 15cm covers 19.1 cm
30% overlap from edges -> 1 spray covers 19.1 – 30% = 13.4 cm



Note: Spray cones may not overlap.
Note: 3 sprays cover 13.4 +13.4 + 19.1 = 45.9 cm
Note: Spraying from below needs to be done under an angle to prevent fouling of the nozzle.

Theoretical coverage over 1 meter line:
100cm - 19.1cm = 80.9 cm. 80.9cm / 13.4cm = 6 → 7 nozzles

Volume displacement for 65º UniJet Spray nozzles, Standard Spray.
At 3 bar, 65º spray nozzles can be purchased with capacities between 0.067 l/min or 4 l/h (code# 0017) and 28 l/min or 1680 l/h (code# 70), see page 2 for more details.
Using a PulsaJet in line with the nozzle can reduce this amount of volume even further.

4. Proposal for industrial trials:

General Application techniques

Method	Pros	Cons	Cap X	Waste
Tumble	Cost effective; controlled	Operator needs to ensure adequate blend time	Low	Low
Spray Bar	Automated	Variability in Coverage	Medium	Medium
Auger	Automated; Excellent Coverage	Larger footprint; moderate waste	Medium	Medium
COPE/Dip	Excellent Coverage	Unused phage left in COPE	Large	Large

Recurrent Application dose:

+/- **1%** PGS solution applied at **1%** pick up level. This suitable concentration to achieve well over 1 $\log_{10}$ kill of *Salmonella* and is currently being used at other customers with comparable applications.

General steps for successful implementation of phage application:

- Check process parameters and other CCP in place on production lines to determine the best point for phage application.
- Measure and note the residual chemistries (PAA, Chlorine, etc.) on product surface if used before (and after if necessary) from the potential point of phage application.
- Prepare phage solution using dechlorinated water (**< 1 ppm** free chlorine present in the

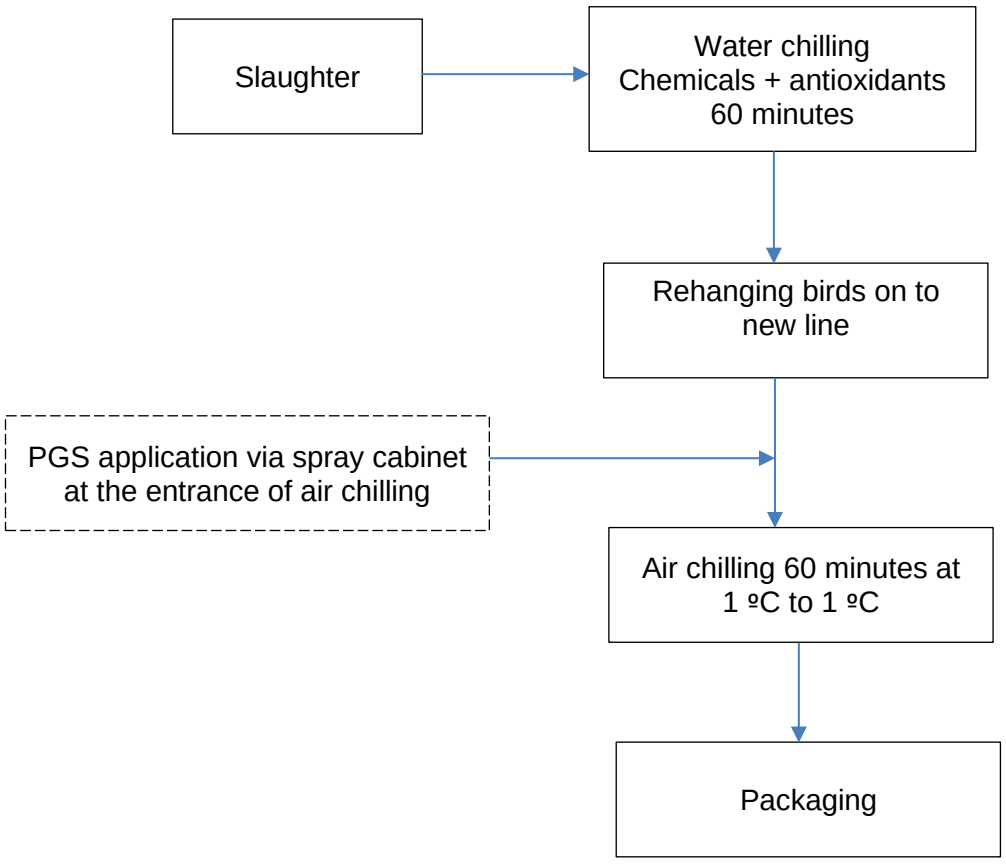
water). If necessary, use in-line chlorine filters to dechlorinate the water.

- Recommend 1 month phage trial. Compare results with historical data, internal samples frequency of *Salmonella* positives.

The poultry industry faces many challenges regarding producing safe foods in a safe environment, phages in addition to use of chemicals in can help with post-harvest poultry processing. PhageGuard S is not a silver bullet nor can it replace all other chemicals or organic acids used in poultry slaughter. When used efficiently and effectively PhageGuard S brings great value on finished products.

PhageGuard S is a natural and organic antimicrobial intervention that kills *Salmonella* by using the natural enemy of the bacteria. It is a safe and powerful tool with many benefits including lack of impact on flavor, odor, texture or protein denaturation of finished products. In addition phages are worker and environmentally friendly. Therefore, PhageGuard S is an effective hurdle against *Salmonella* during processing of fresh meats, leading to safer products without compromising worker safety.

Example Agrosuper Process overview:



Proposition for phage application in the Agrosuper process:

Note: PGS volumes calculated are for one full production day (4 processing lines).

Application point carcass: Spraying carcass at the entrance of air chiller
Proven application: South Africa customer trail spray on poultry carcass
Volumes to carcass: Surface area of carcass: ~ 1,809 cm^2 W = ~ 1.9 kg (weight carcass, whole gutted and head off) Quantity per day: ~ 176,000 chickens/day (22,000 per hour, 8 hours of production)
PGS conc: 0.5%
Pick up: 10 µL/cm^2 of surface area of carcass (~18-20 mL spray per carcass)
PGS units: ~16 L /day
Total PGS working solution: ~3,184 L/day

General preparation and storing of PGS working solution:

Formula to calculate the amount of PGS units (L) needed to make working solution:

$$\textit{Amount of undiluted PGS (L)} = \textit{volume of work solution needed (L)} \times \frac{\textit{PGS concentration recommended (\%)}}{100}$$

Steps to make working solution:

1. Rinse the container thoroughly (plastic / stainless steel) with chlorine free water. Drain the water out completely.
2. Fill exactly XXXX Liters of chlorine free tap water (temperature 5 – 20 °C). Note: insert a chlorine filter removal system inline of the stream if needed to reduce Cl levels to 0 ppm. Contact Micreos personnel if you need assistance with this step.
3. Add XX Liters of undiluted PhageGuard S (calculated using the above formula) and stir gently using a clean mechanical/manual stirrer (1 minute, low speed). Short mixing helps with equilibration of the working solution.
4. After preparing use immediately. In case of later use, store the working solution at temperatures < 12 °C for up only up to 18 hours.

5. It is highly recommended to make fresh working solution for every new production shift.
6. Do not store or use working solutions that is stored for more than 24 hours.
7. Spray the working solution on the exterior of the product to treat. Ensure all relevant surface areas of the products are treated appropriately

5. Conclusion

The Poultry Industry faces many challenges regarding producing safe foods in a safe environment and phage can help replace or reduce the use of chemicalsin post-harvest poultry processing.

PhageGuard S is a natural and organic antimicrobial intervention that kills *Salmonella* by using the natural enemy of the bacteria. It is a safe and powerful tool with many benefits including lack of impact on flavor, odor, texture, and protein denaturationof finished products. In addition, Phage is worker- and environmentally friendly. PhageGuard S is an effective anti-*Salmonella* hurdle during processing of fresh meats, leading to safer products without compromising worker safety.

Though phages work on rough surfaces such as carcasses and large surfaces relative to weight, such as minced meat. The most ideal application would be chicken fillets, due to them having a smooth, small surface relative to weight, making the intended application of phages on chicken fillets is a favorable one.

- Smooth surface, allowing for great distribution at lower relative volumes.
- A high value product where organoleptics (odor, taste, texture, color) are of significant importance.

PhageGuard S is not a silver bullet, nor can it replace all other chemicals or organic acids used in poultry slaughter. However, when used efficiently and effectively on finished products it increases poultry product value and quality. Mitigating exposure to risk.

(1) *Effect of ultraviolet light, organic acids, and bacteriophage on Salmonella populations in ground* Yeh Y, de Moura FH, Van Den Broek , de Mello AS (January, 2018) .

(2) *Population Dynamics of Salmonella enterica Serotypes in Commercial Egg and Poultry Production.* Steven L. Foley, Rajesh Nayak, Irene B. Hanning, Timothy J. Johnson, Jing Han and Steven C. Ricke

(3) *How Foster Farms Is Solving The Case Of The Mystery Salmonella*, NPR Morning Edition Transcript (August, 2014)

(4) *Salmonella Typhimurium DT 104 response to Lytic bacteriophage and Lactobionic acid on raw chicken breast. Nicole Walker, Sherita Li, Hannah Strauss, Siroj Pokharel (June, 2021)*

6. Appendix

Appendix 1: The 3 D's for application success

The 3 D's for implementation success.

- Dose (or Concentration)
- Distribution
- Dwell time (or Contact time)

Appendix 2: Micreos company overview

Under the PhageGuard brand Micreos has also developed FDA-approved food safety products against Salmonella, Listeria monocytogenes and E. coli. More information is available at the PhageGuard website.

The company is committed to helping mankind with products that are effective, sustainable, and affordable. The company's products:

- work fast
- are easy to apply
- have no known adverse effects

Micreos Technology Centre Zurich is based in Wädenswil, close to Zurich. Micreos Pharmaceutical Development Team and manufacturing plant are based in Bilthoven at the RIVM premises (Dutch NIH). Micreos Food Safety is based in Wageningen 'Food Valley'. Micreos headquarters are located in The Hague, world capital of Peace and Justice.

We work according to the highest ethical and professional standards, as witnessed by numerous awards and grants received over the past years, and publications in scientific journals and regular press. Micreos collaborates with top institutes and companies around the world and its advisors and partners have broad expertise in the fields in which we operate. Our long-term research partners include ETH Zurich, Switzerland, (Prof. Loessner) and Erasmus Medical Center Rotterdam (Prof. Pasmans, pediatric dermatology).

Dutch company; Global leader in applied phage technology
US sales office & stock position
Targeted anti-bacterial solutions
Phages have applications in: Human Health MR(SA), Food Safety, Animal Health.

glad skin
bacterial skin balance

PhageGuard
FOOD SAFETY POWERED BY NATURE

Bilthoven – Human Health

Wageningen – Micreos Food Safety