

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

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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.

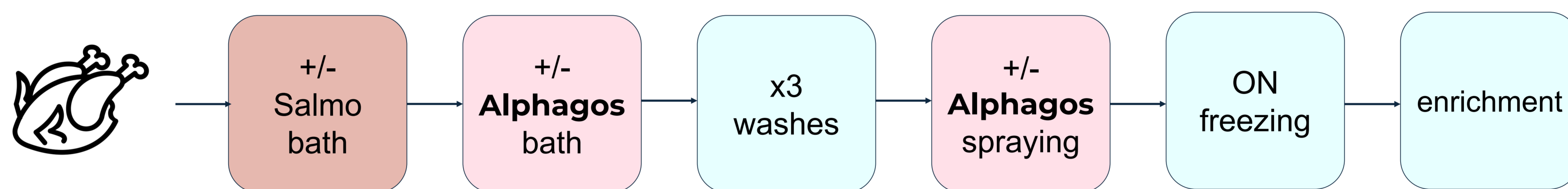
Cocktail design

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	<i>Salmonella</i> 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

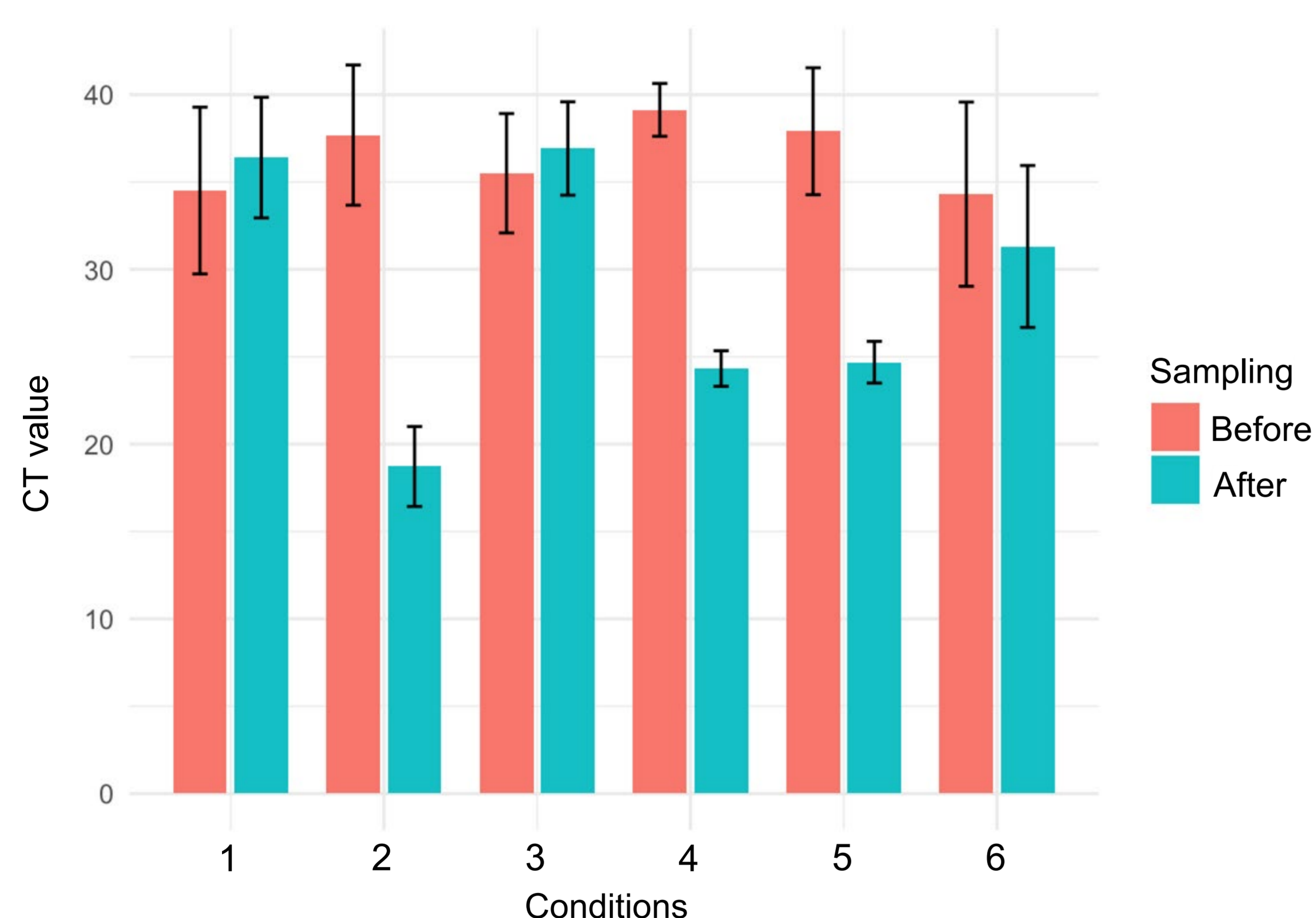


Figure 1 - Mean CT values obtained from qPCR analysis for six different conditions, measured both before (in red) and after (in green) the carcass processing. The qPCR was performed using the FoodProof detection kit targeting *Salmonella enterica*. Each bar represents the mean CT value from two biological 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**.