

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



Ilias Theodorou*¹, Marine Feyereisen*¹, Fitri Luthfianti Nur Annisaa², Irfan Refangga², Houa Oukaci¹, I Wayan Wisaksana Yasa², Inna Herliana², Adèle James¹

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	<i>Salmo</i> 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

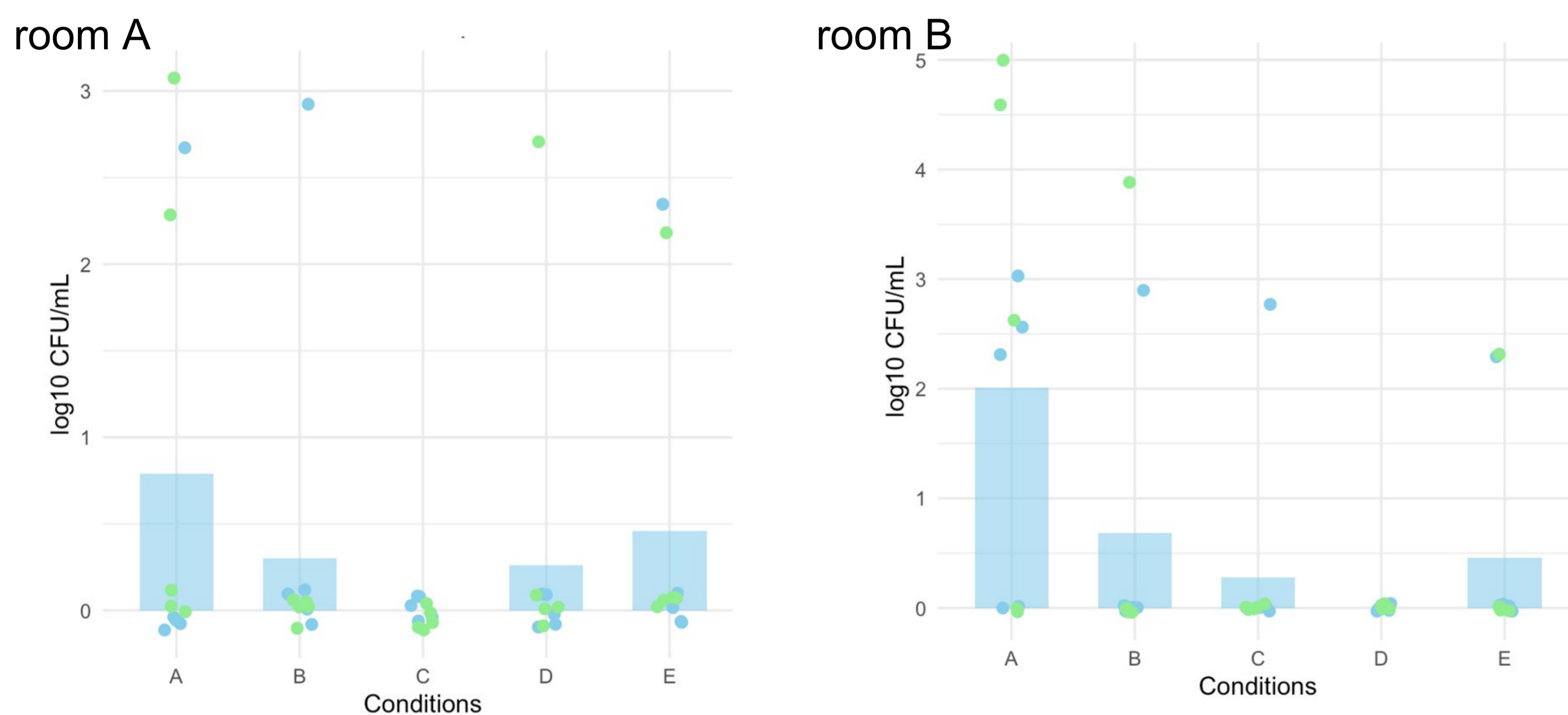


Figure 1 - Mean of the *Salmonella enterica* CFU counts on XLD expressed in log₁₀ CFU/mL of organs resuspended in sterile PBS in a weight:volume 1:1 ratio for each condition of the trial. The bars represent the mean *Salmonella enterica* CFU counts/condition. Each count (5 animals x2 cages) is represented 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**.