Use of bacteriophage as an antimicrobial intervention against *Salmonella* in poultry

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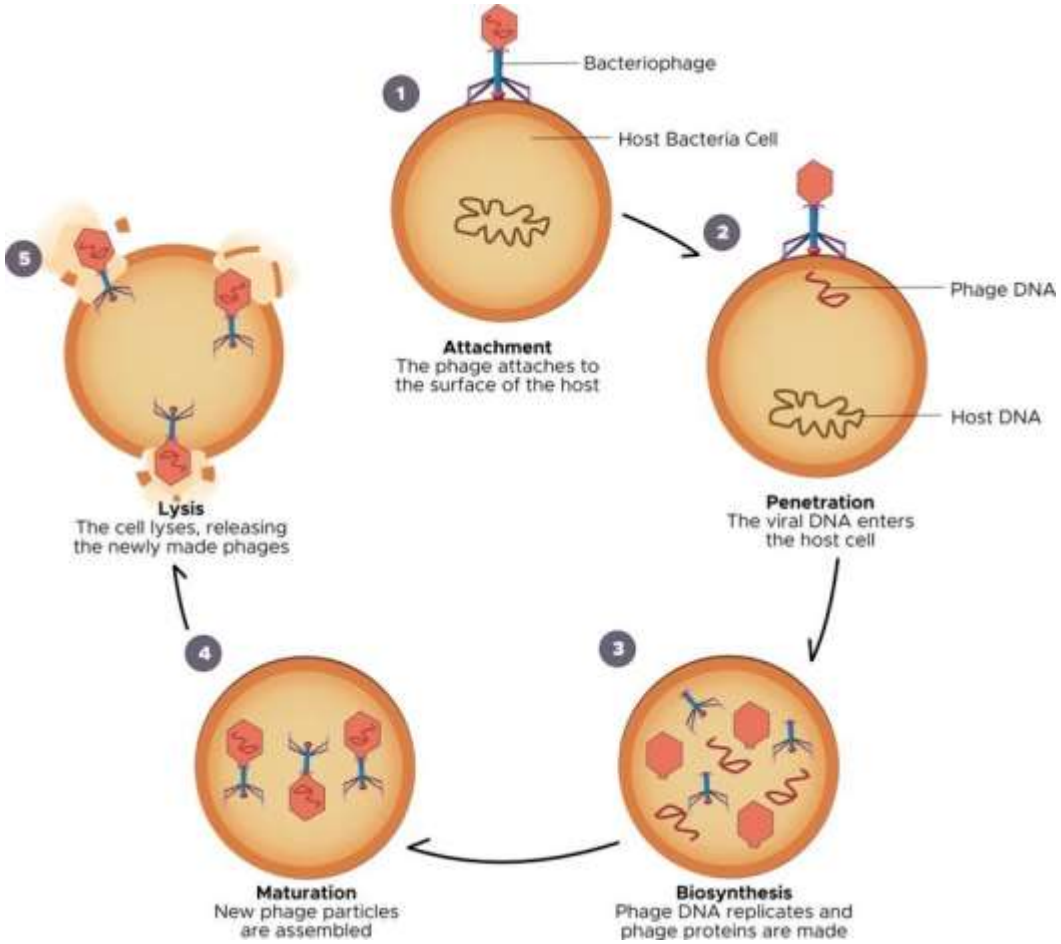
Outline

- **Intervention details**
  - **Bacteriophage definition and advantages to its use**
- **Validation study for phage use in poultry**
  - **Benchtop considerations prior to scale-up**
  - **Product testing to inform strategic phage**
  - **Validation sample set results**
  - **Extended routine sample set results**

Intervention Details

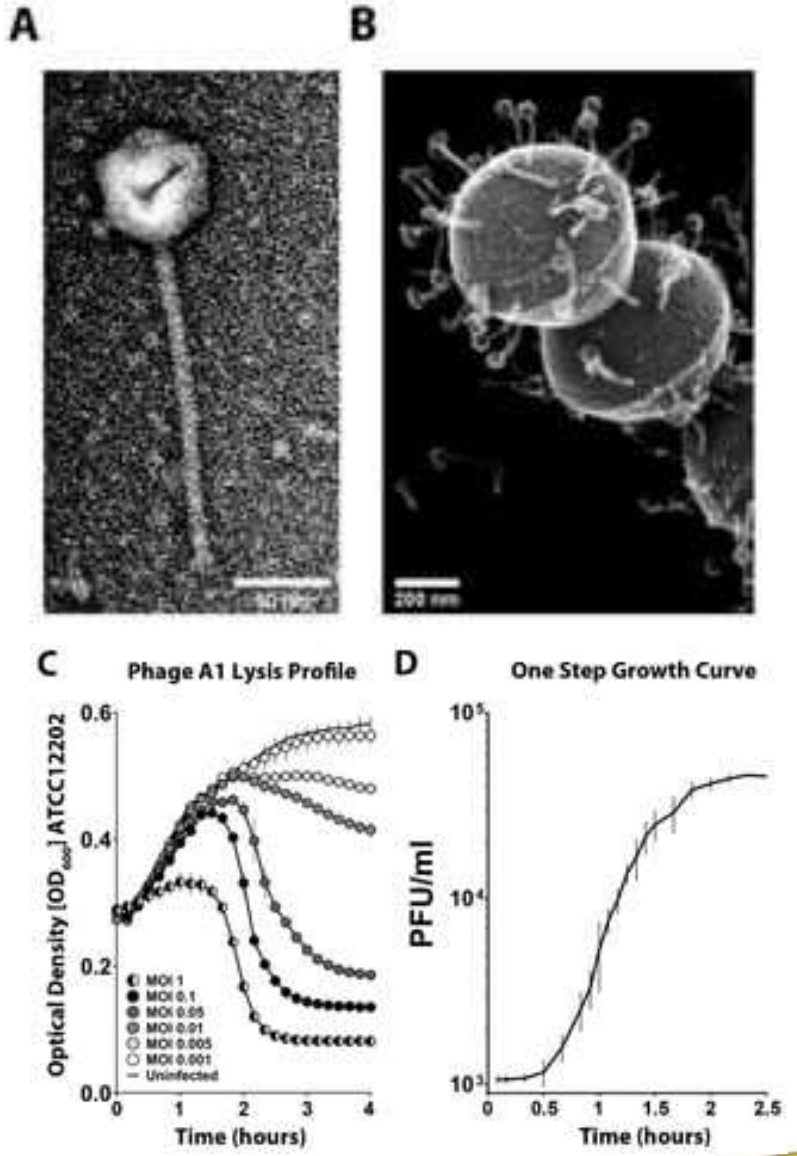
- Bacteriophage (lytic)
  - Virus that attaches, infects, and inactivates bacteria
  - Ubiquitous in the environment
  - Specific host range (i.e., targeted approach for pathogen reduction)
  - GRAS status
  - Natural and safe for consumers
  - No label requirements when used under accepted conditions for use



AIMS Microbiol. 2020;6(3):204-230.

Benchtop considerations prior to validation study

- Understand serotype/subtype distribution of target organism within your system
- Understand concentration of target organism in your product
  - ≤ 10CFU/g Salmonella in raw ground comminuted poultry
  - Phage multiplicity of infection (10,000:1)[1]
- Plaque assay/spot assay to validate phage solution is effective against serotypes in your system
- Water hardness and presence of residual disinfectants
  - Ca^{2+}, Mg^{2+} for virion-host cell attachment[2]
  - Chlorine from municipal water inactivate phage



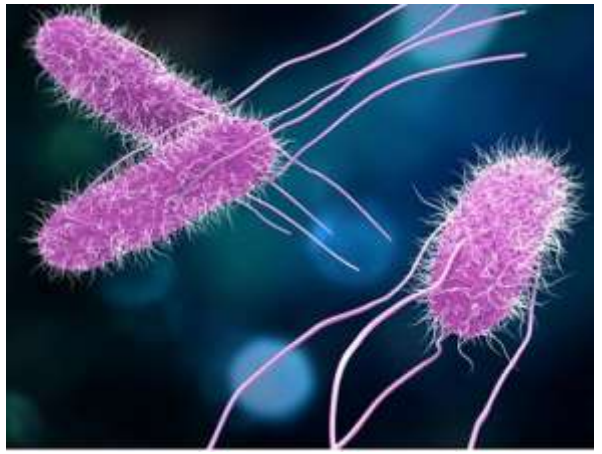
[1]Poultry Science (2017): 96:2845–2852

[2]Mol. Biol. Rep (2014), 41: 5829-5838

Viruses (2021): 13, 612

Intervention Validation

- Objective: Assess phage treatment efficacy by the treatment of whole part meat inputs via a dip tank application
  - Meat input chosen based on historical prevalence of ground samples with identified input
- Dip tank application chosen to provide best coverage of targeted whole part meat inputs
  - Air agitation ensure constant mixture at target concentration within the dip tank application
- Validation Method:
  - Validation Study: Samples were analyzed for *Salmonella* at the following stages:
    - Pre-Treatment (whole part)
    - Post-Treatment (whole part)
    - Post-Treatment 2hr hold (whole part)
    - Post Grind (ground)
  - Pre-Implementation vs. Post Implementation: Data was compared across time periods for all routine ground samples
    - Data was broken down into 3 categories:
      1. Ground samples with only the treated meat input
      2. Ground samples with the treated meat input alone or in combination with other inputs
      3. Ground samples without the treated meat input



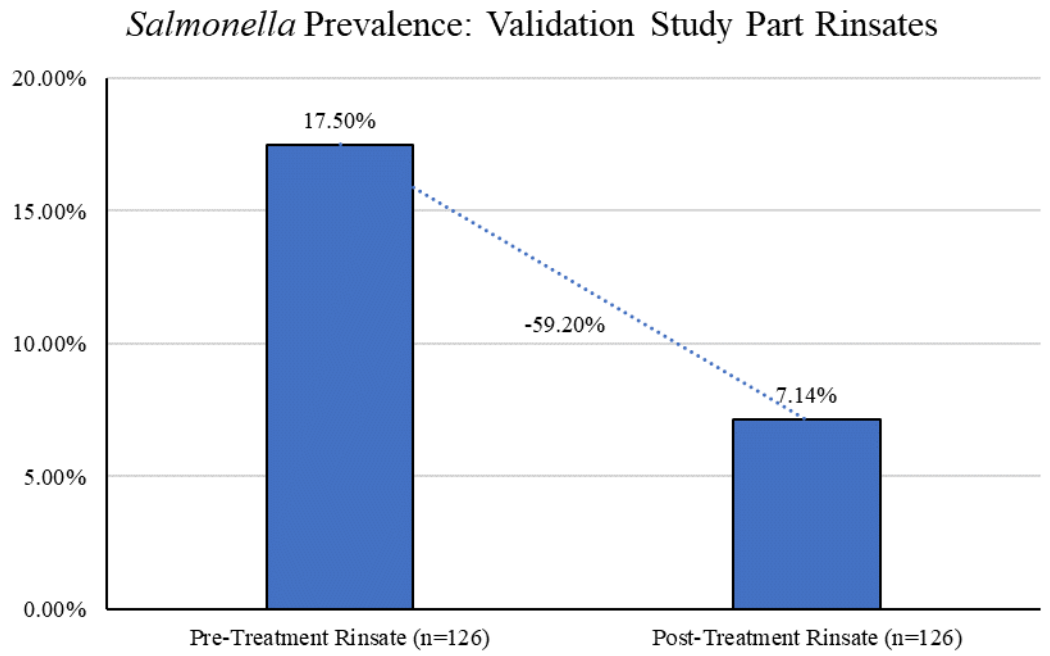
https://www.fda.gov/food/foodborne-pathogens/salmonella-salmonellosis

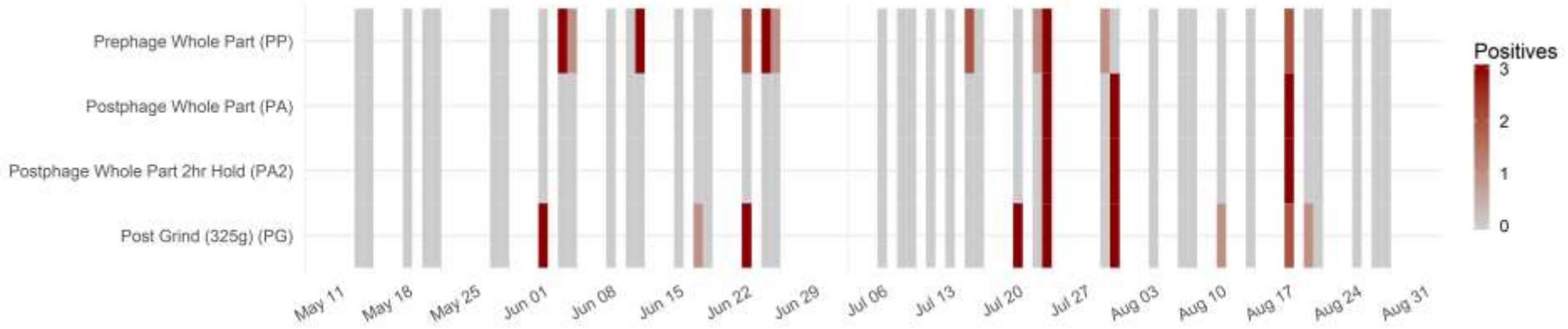
Phage Dip- Validation Sample Set

Salmonella Prevalence Summary

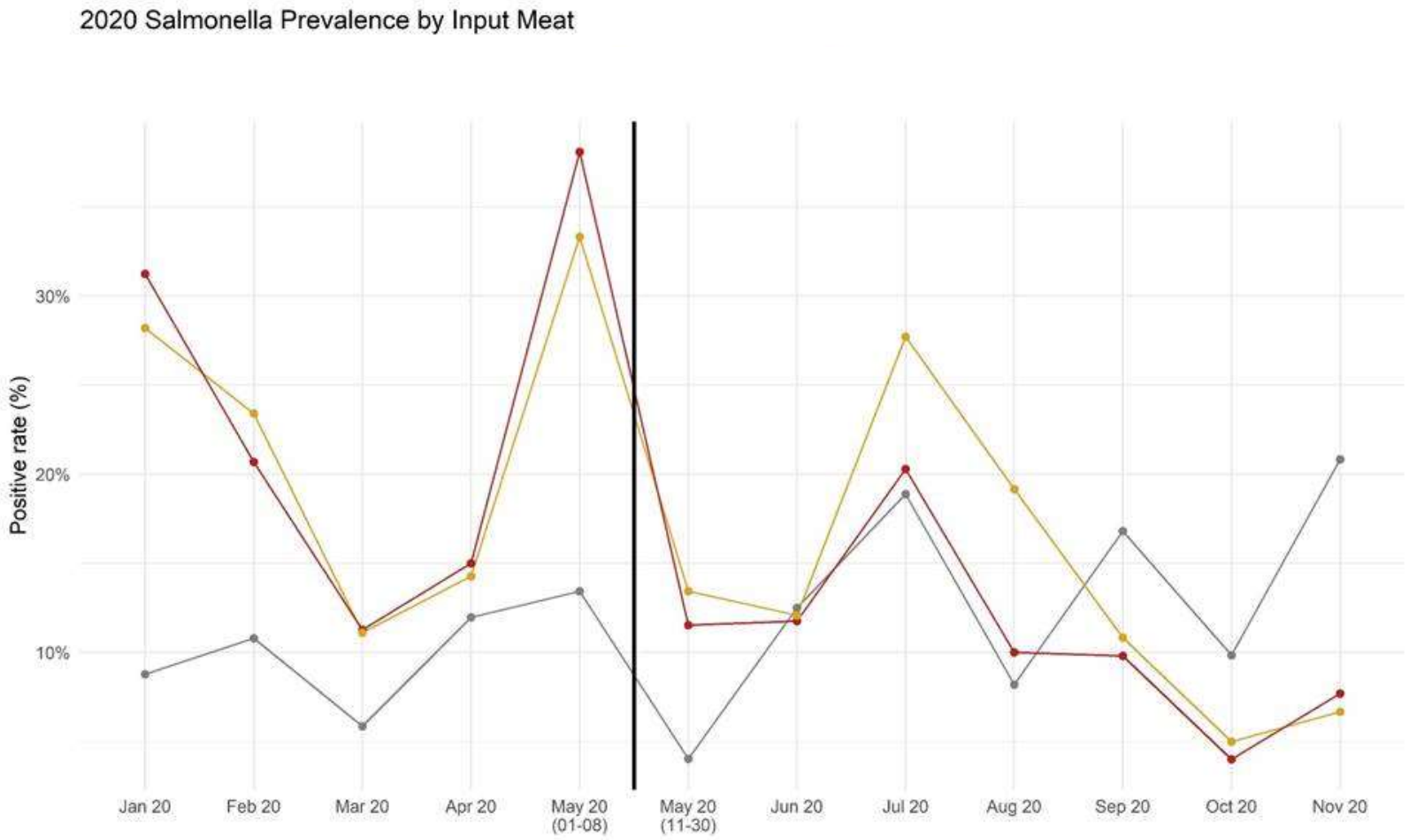
Sampling Location	Positive Rate (%)
Prephage Whole Part	17.5
Postphage Whole Part	7.14
Postphage Whole Part (2 hr hold)	7.14
Post Grind	15.9

126 samples per location, 42 sampling days
All Quant values are <1CFU/ml or g





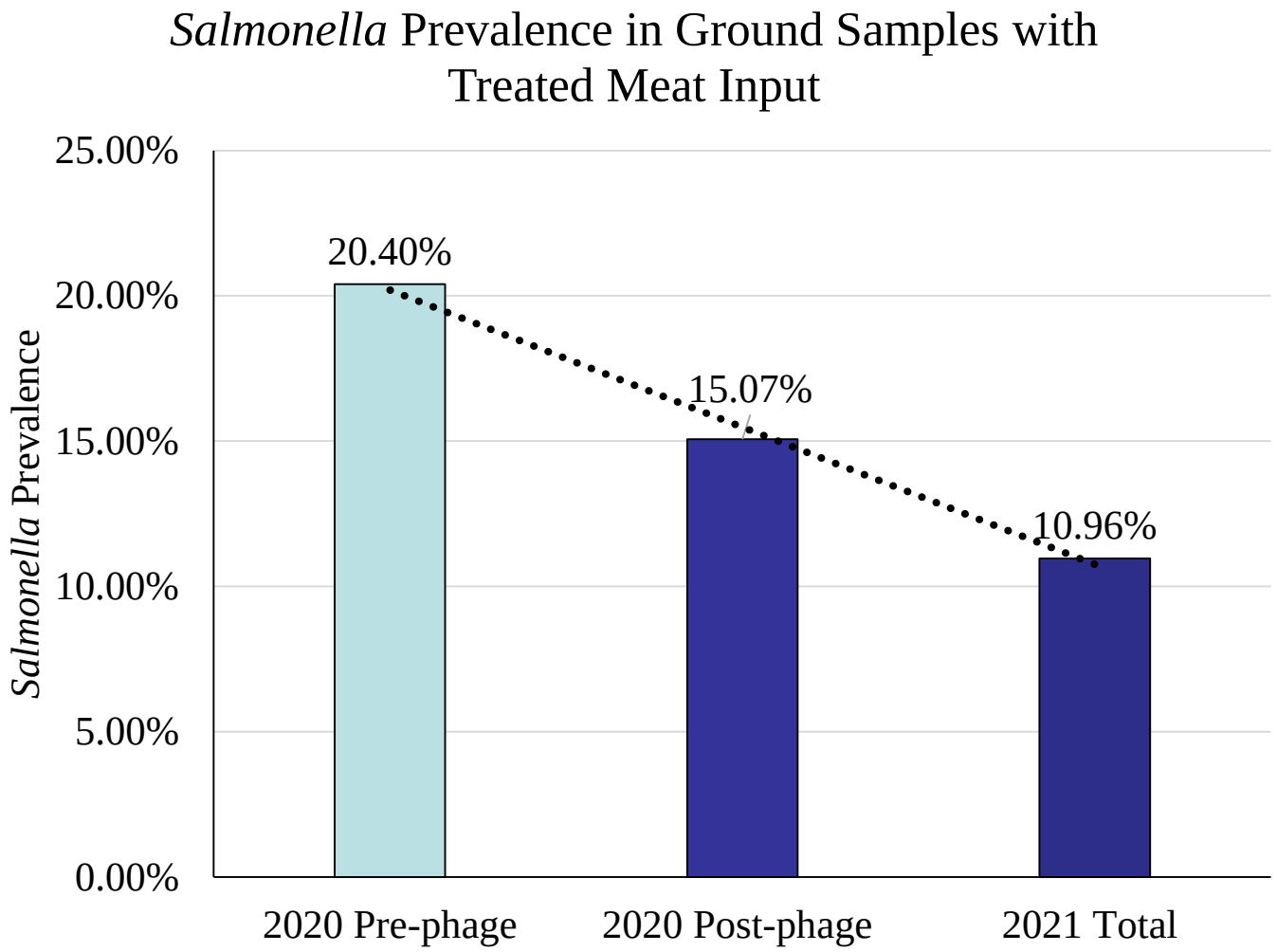
Phage Dip Trial- Routine Sample Set



- Ground samples with treated meat input (both alone- red and in combination with other meat inputs- yellow) in relation to prevalence in samples without treated meat input for the same time period
- Historically samples with treated meat input have shown a greater *Salmonella* prevalence
- Following implementation of the phage dip ground input samples with treated meat input have shown a marked decrease in prevalence to levels equal to and below that of other samples

Continued Efficacy

- Continued efficacy in ground samples with the treated meat input (both alone and in combination with other inputs) has been illustrated since the implementation of the dip application
- 2020 Pre/Post implementation illustrated ~26% reduction in *Salmonella* prevalence
- 2020 Pre-implementation – 2021 illustrated a ~46% reduction in *Salmonella* prevalence



Conclusion

- During the validation study *Salmonella* prevalence in whole part rinsates was significantly lower following phage treatment (~-59%) in samples collected both immediately after treatment and held of 2 hours
- Ground samples with treated meat input only showed the largest change in *Salmonella* prevalence (~43%) between Pre/Post-implementation time period in 2020
- Ground samples with treated meat input alone or in combination with non-treated inputs illustrated a reduction of ~26% in *Salmonella* prevalence between Pre/Post-implementation time period in 2020
- Continued reduction illustrated between 2020 Pre-implementation through 2021 (~46%)
- Phage treatment of whole parts via dip application demonstrated a feasible approach to targeted *Salmonella* mitigation in both whole parts and ground poultry samples