

Efficacy of bacteriophage mediated colibacillosis control in laying hens



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Introduction

- Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis in laying hens¹.
- Colibacillosis results in significant economic losses to the poultry industry².
- Bacteriophage (phage) therapies have been effective in reducing respiratory disease caused by APEC in broilers³.
- Here we report data on APEC phage prevalence in hen feces and sewage, as well as the characterization of seven anti-APEC phages.

Hypothesis

Since anti-APEC phage cocktails reduce colibacillosis infection in broilers, phage cocktail application should improve hen health outcomes when applied post-APEC exposure.

Objectives

1. Characterize novel anti-APEC phages isolated from hen feces and sewage water.
2. Utilize phage cocktails to control APEC infection in a laying hen model.

Methods and Materials

- **Bacteria:** O1, O2 and O78 APEC clinical isolates from chickens.
- **Phage Isolation/purification:** Phages were isolated from hen feces and sewage water, and purified using standard methods.
- **Phage virulence assay:** Microplates containing 10-fold phage serial dilutions and overnight APEC culture were incubated at 37°C for 5 hours to determine the multiplicity of infection (MOI) by assessing APEC culture clearing⁴.
- **Transmission Electron Microscopy (TEM):** Phages were imaged by TEM using standard methods

Results and Discussion

- 28 phages with anti-APEC activity were isolated between May and September 2020.
- 7 phages were chosen for further characterization based on their ability to propagate in APEC culture.
- Phage plaque morphologies were mainly clear and circular though some variation was present (Figure 2)
- Results suggest phages targeting APEC are abundant in hen feces, but may be difficult to propagate in pure culture.

- Three phages have been imaged via TEM following propagation.
- All phages were tailed-phages possessing an icosahedral capsid.
- 2SW2 is an SfiV-like phage of the *Myoviridae*, 3SW1 is a Vil-like phage of the *Myoviridae*, and 6SW2 is a phage of the *Siphoviridae* family.

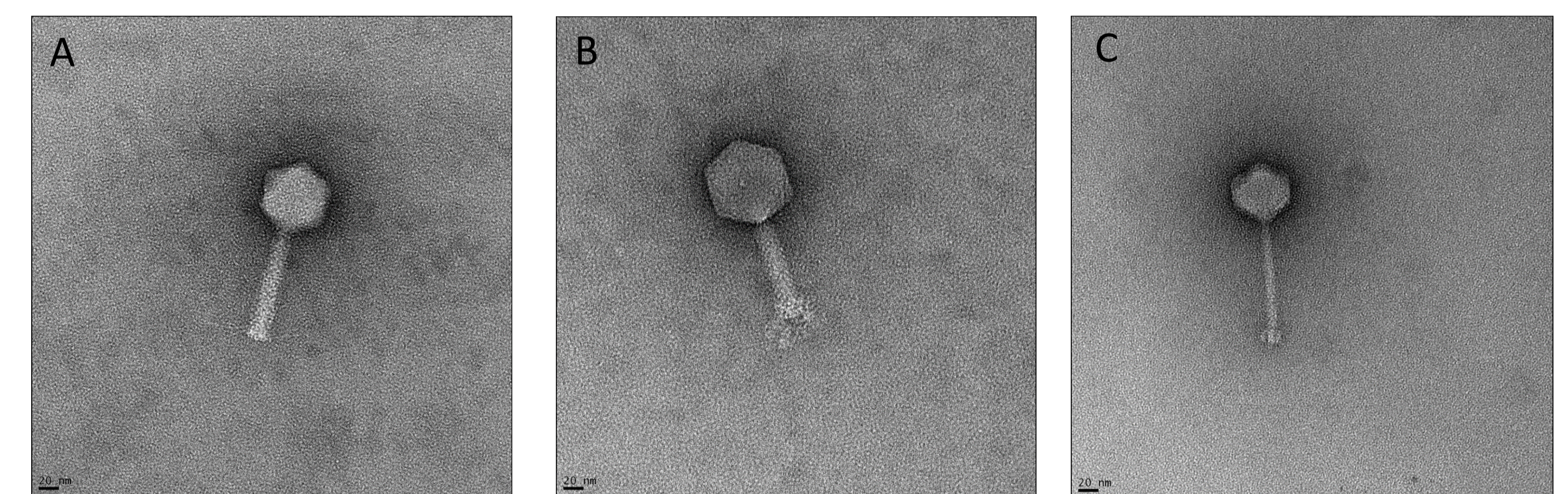


Figure 3. TEM imaging of phages A) 2SW2 B) 3SW1 and C) 6SW2

- The APEC host range and lytic ability of 7 phages was determined via a microplate phage virulence assay.
- All phages tested were able to lyse at least two clinical APEC strains, with 5 phages exhibiting cross-serogroup efficacy.
- Phages isolated using O78 *E. coli* as a trapping host had greater activity against O78 APEC strains, though SW1 possessed lower activity against all tested APEC serogroups.
- Overall, results suggest that phages SW1, 6SW2 and 6VE1 show promise for the treatment of colibacillosis caused by O78 APEC strains.

| APEC Strain | Phage Strain | | | | | | |
|----------------|--------------------|--------------------|--------------------|--------------------|------------------------|------------------------|--------------------|
| | 2SW1 | 2SW2 | 3SW1 | 3SW2 | SW1 | 6SW2 | 6VE1 |
| ECL20834 (O1) | Resistant | Resistant | Resistant | Resistant | Moderately Susceptible | Resistant | Resistant |
| ECL20885 (O1) | Highly Susceptible | Highly Susceptible | Highly Susceptible | Highly Susceptible | Moderately Susceptible | Resistant | Resistant |
| ECL21443 (O2) | Highly Susceptible | Highly Susceptible | Highly Susceptible | Highly Susceptible | Highly Susceptible | Resistant | Resistant |
| ECL22102 (O78) | Resistant | Resistant | Resistant | Resistant | Highly Susceptible | Moderately Susceptible | Highly Susceptible |
| ECL20719 (O78) | Resistant | Resistant | Highly Susceptible | Resistant | Highly Susceptible | Highly Susceptible | Highly Susceptible |
| ECL23026 (O78) | Resistant | Resistant | Resistant | Resistant | Highly Susceptible | Highly Susceptible | Highly Susceptible |

Legend: Extremely Susceptible (1E-08sMOI/0.01), Highly Susceptible (0.01sMOI/1), Moderately Susceptible (1sMOI/10), Minimally Susceptible (10sMOI/100), Resistant (100sMOI)

Figure 4. Host range and lytic activity of 7 anti-APEC phages.

Fecal and sewage sample phages targeting *E. coli* O1, O2 and O78 serogroups

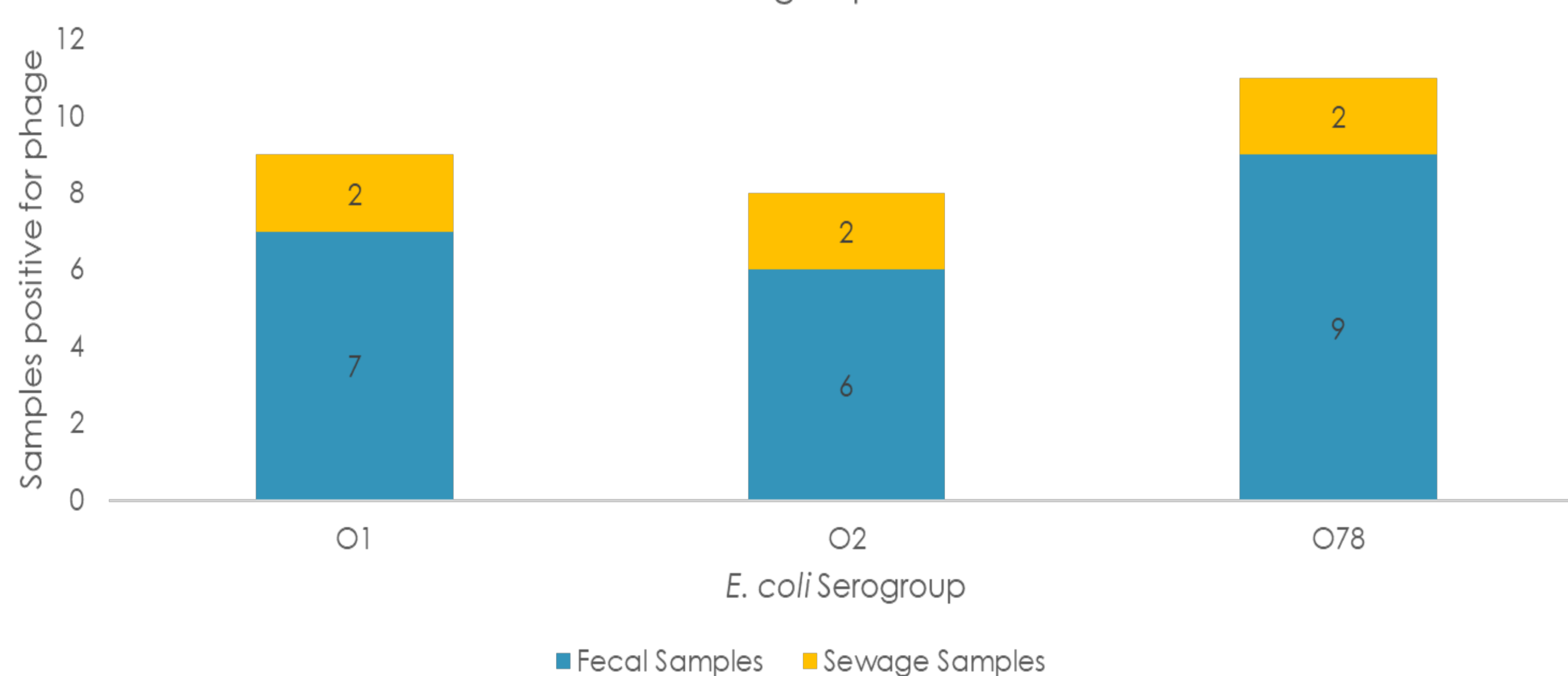


Figure 1. Fecal and sewage samples positive for phage targeting O1, O2 and O78 *E. coli*.

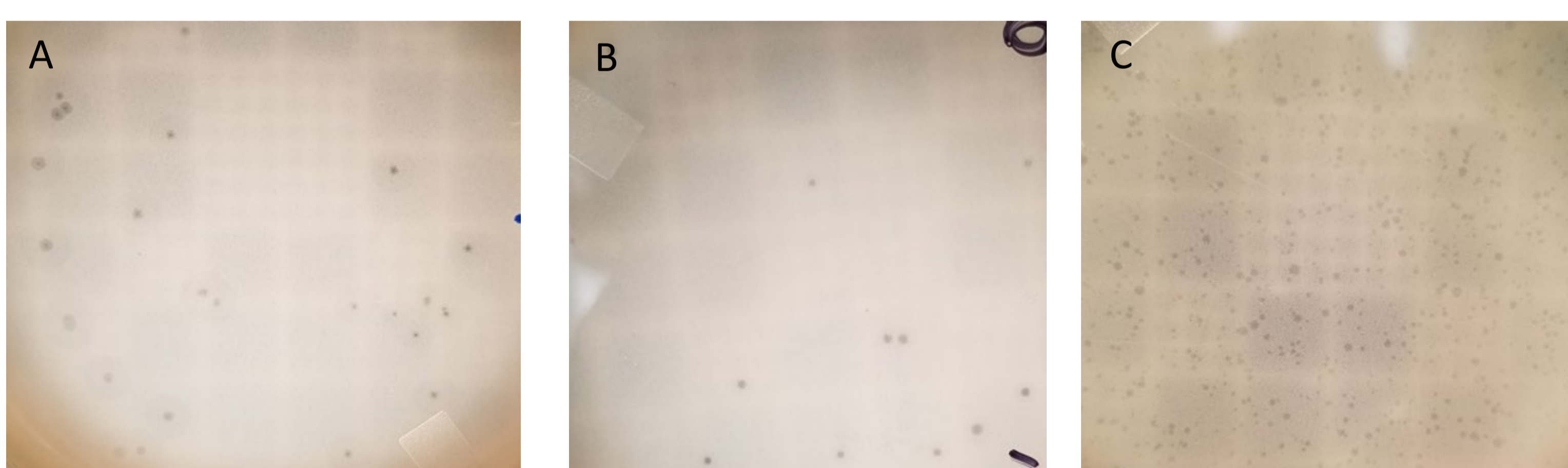


Figure 2. Phage plaques produced from enriched fecal filtrates and sewage. A) 6SW2 B) 6VE1 C) F-01-1

Future Directions

- Determine the lytic activity of phage cocktails containing the three above phages for use in future *in vivo* studies.
- Determine infectious potential of laboratory APEC strains in laying hens.
- Assess the efficacy of phage cocktails in improving colibacillosis outcomes in laying hens when administered via: Environmental sprays, water inclusion, and intramuscular injection.

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Literature Cited

1. Kabir SML. 2010. Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns. *Int J Environ Res Public Health* 7(1): 89-114. DOI: 10.3390/ijerph7010089
2. Landman WJ, van Eck JH. 2015. The incidence and economic impact of the *Escherichia coli* peritonitis syndrome in Dutch poultry farming. *Avian Pathol* 44:370-8.
3. El-Gohary FA, Huff WE, Huff GR, et al. 2014. Environmental augmentation with bacteriophage prevents colibacillosis in broiler chickens. *Poult Sci* 93:2788-2792. DOI: 10.3382/ps.2014-04282.
4. Niu YD, Johnson RP, Xu Y, McAllister TA, Sharma R, Louie M, Stanford K. 2009. Host range and lytic capability of four bacteriophages against bovine and clinical human isolates of Shiga toxin-producing *Escherichia coli* O157:H7. *J Appl Microbiol* 107(2): 646-656. DOI: 10.1111/j.1365-2672.2009.04231.x.