Use of bacteriophage Felix01, HL18 and HL03 to reduce *Salmonella enterica* Typhimurium burden in mice.

A.S. Leaflet R1975

N.M Boury, lecturer of animal science
N. K. Lee, graduate assistant
D. L. Harris, professor of animal science and veterinary diagnostic and production animal medicine.

Summary and Implications

Multi-drug resistant (MDR) strains of *Salmonella enterica* serovar Typhimurium are of increasing concern in the food industry and on the farm. While these strains are becoming increasingly resistant to commonly used antimicrobial agents, they remain sensitive to killing by their natural predators, bacteriophage. Bacteriophage are able kill MDR strains of Salmonella in vitro. We have recently tested the ability of a well-known salmonella bacteriophage, Felix 01 and two recently isolated phage (HL03 and HL18) to reduce the *Salmonella Typhimurium* burden in orally challenged, susceptible mice. When each of the phage was given at the same time as the oral Salmonella challenge, they significantly reduced the Salmonella burden in the treated mice. This lowering of Salmonella load occurred when the phage were given at a 1:10, 1:50 and 1:100 MOI when compared to the bacterial challenge dose. Interestingly, of the three phage tested (Felix, HL03 and HL18), only the last phage, HL18 was effective when given an hour before or an hour after Salmonella challenge. Felix01 and HL03 were both ineffective when given an hour before or an hour after challenge, but consistently lowered the bacterial burden in these mice when given at the same time as the challenge dose. These data indicate that bacteriophage-based therapy may be an alternative to antibiotic-based treatments to lower the Salmonella levels in swine and potentially limit the spread of Salmonella during transport and lairage of swine prior to slaughter..

Introduction

Foodborne salmonellae causes an estimated 1.4 million cases of salmonellosis each year in the United States alone. Recent studies have indicated that a significant number or retail ground meat sources, such as ground chicken, turkey, pork and beef contain various serovars of *Salmonella enterica*. When these bacteria isolated from retail meat sources were further tested, researchers found that 84% of them were resistant to at least one antibiotic and 53% were resistant to three or more antibiotics, including those antibiotics commonly used to treat salmonellosis. If infected with multi-drug resistant (MDR) Salmonella, people are significantly more likely to be hospitalized and to stay in the hospital longer than those people infected with antibiotic-susceptible Salmonella.

One promising method to control MDR strains of bacteria is the use of lytic bacteriophage to kill bacterial cells. This method has been shown to be effective against group A streptococcal infection, *Vibrio vulnificus* and *Enterococcus faecium* in mice and against *E. coli* infection in chickens. We have recently isolated two environmental bacteriophage that are able to lyse multidrug resistant Salmonella in vitro. In this study we tested these two phage with a well characterized lytic bacteriophage, Felix01 to determine the phage's ability to decrease the burden of MDR *Salmonella Typhimurium* in susceptible mice.

Materials and Methods

Each phage (HL3, HL18, and FO) was cultured overnight with Salmonella in GCA medium. The culture was centrifuged for 30 minutes at 4C to remove bacterial debris and the supernatant phage lysate was harvested. Phage lysate was filtered through a 0.22um disposable filter and stocks were kept at 4C. The concentration of phage particles was determined by plaque assay and expressed as plaque forming units (PFU) per milliliter.

For the mouse model of salmonellosis we used an oral challenge model. Briefly, male C57BL6 mice were inoculated via gastric feeding needle with approximately 10^7 CFU/mouse of Salmonella X4232 NalR GFP in 0.1 mL of PBS. The mice were then treated with either PBS (negative control), live phage (either HL03, HL18 or Felix 01) or autoclaved phage (killed phage control). Bacteriophage (Felix01, HL18 or HL03) were given at a concentration of 108 PFU/mouse at the same time the mice were inoculated with 107 CFU/mouse *Salmonella Typhimurium* (X4232). In dose titration experiments, the mice were given 107 CFU/mouse *Salmonella Typhimurium*, and varying doses of each of the three bacteriophage. Four hours after inoculation with Salmonella, the mice were killed by CO2 administration and the cecum and intestine were harvested from each. Tissues were homogenized, homogenates were diluted out and plated onto XLD plates containing naldixic acid and onto blood agar plates (to assure no Nal-susceptible contaminants existed). Data are expressed as CFU/gram of tissue. .

Results and Discussion

As shown in Figures 1 and 2, Felix 01 administration significantly reduced the levels of *Salmonella Typhimurium* in inoculated mice. When given at an MOI of 10:1 or 100:1 PFU:CFU ratio, Felix was able to reduce bacterial the burden of Salmonella in the intestines and ceca of infected mice. This reduction was not seen at a 10:1 MOI if the

phage treatment was given one hour before or one hour after Salmonella challenge.

Figure 1. Dose response of Felix01 against murine salmonellosis.

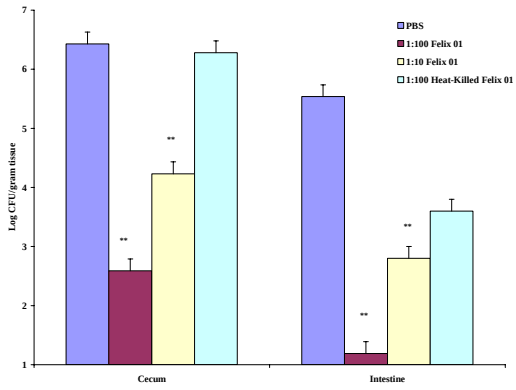
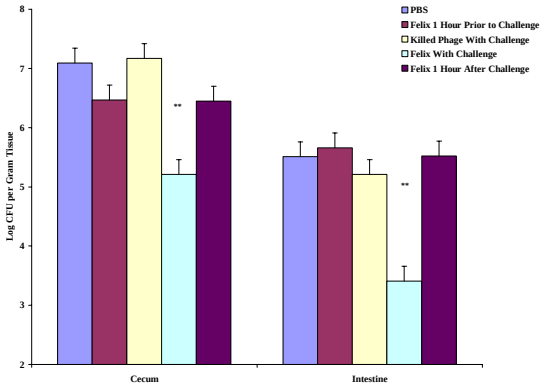


Figure 2 Timing of Felix01 administration determines its efficacy in vivo.



We saw similar, but distinctive results with similar experiments testing the lab isolate, HL18. As shown in Figure 3, the HL18 bacteriophage significantly reduced the levels of Salmonella in the tissues tested at an MOI as low as 1:1. Also of note, a 1:10 dose of HL18 was able to significantly lower the Salmonella load in the intestines and ceca of mice treated before, after or at the same time as the inoculation (Figure 4).

Figure 3. Dose response of HL18 treatment of oral salmonellosis in mice.

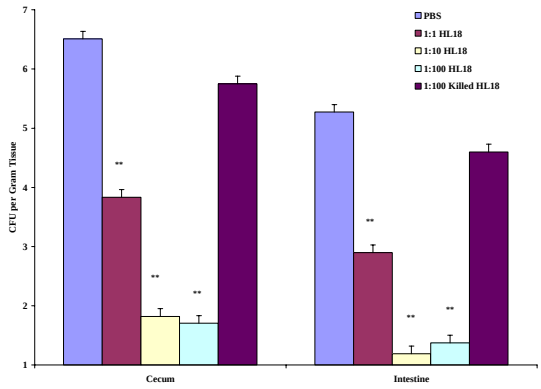
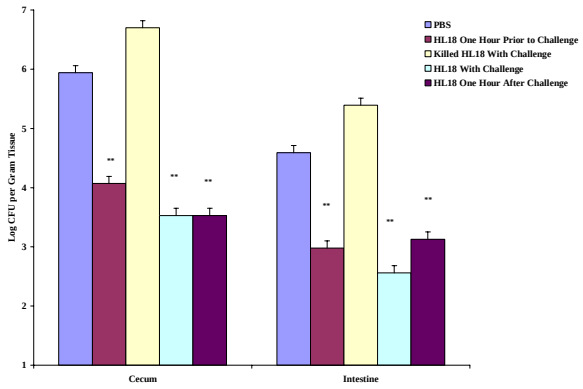
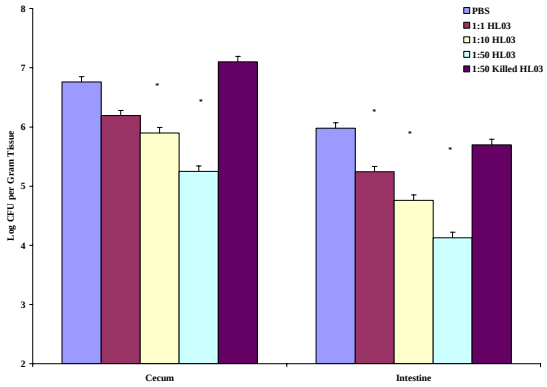


Figure 4. Effect of timing on the efficacy of HL18 administration.



The same challenge experiments using HL03 to treat the mice showed that HL03 was less effective against *Salmonella Typhimurium in vivo*. The dose-response curve (Figure 5) showed r an MOI of 1:50 was required to significantly reduce the Salmonella levels in the ceca of infected mice.

Figure 5. Dose response of HL03 treatment of oral salmonellosis in mice.



These data indicated that while both Felix01 and HL18 could lower Salmonella counts in the alimentary canal of the mice, HL03 required higher doses for similar effects and did not consistently reduce Salmonella levels in mice, even when given in 10-fold excess of the challenge dose. Upon comparing HL18 and Felix01 it was discovered that the environmentally isolated HL18 bacteriophage was effective at lower concentrations and had a longer time range in which it could be administered to still see an effect. Preliminary *in vitro* work on other MDR Salmonella serovars shows that HL18 is effective against them as well. Taken together, these data provide evidence for an effective strategy to limit the growth of Salmonella, even those Salmonella strains resistant to multiple commonly used antimicrobial agents.

Acknowledgments

The authors would like to thank, the Biotechnology Research and Development Corporation for their financial support. The technical assistance of Kay Christiansen, Stephen Gaul, Brad Criswell, Abbie Skyles, and Brenda

Crabtree is greatly appreciated. The authors would additionally like to thank Burt Augustin, Tracy Rullestad, and Pam Whitson for their time and assiduous care of the animals, and Christian Baum for providing the *Salmonella* Typhimurium GFP challenge strain