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Critical Evaluation of Bacteriophage to Prevent and Treat Colibacillosis in Poultry

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Abstract

There is a continuing need to find alternatives to antibiotics in animal and human medicine. Bacteriophages are viruses that infect and kill bacteria, with no known activity to plant and animal cells. We have conducted research to critically evaluate the efficacy of bacteriophage to both prevent and treat colibacillosis in poultry. Bacteriophages lytic to an *Escherichia coli* pathogenic to poultry were isolated from municipal waste water treatment plants and poultry processing plants. Two bacteriophage isolates were selected to use in studies designed to determine the efficacy of these bacteriophage to prevent and treat severe colibacillosis in poultry. Colibacillosis was induced by injecting 6 X 10^4 cfu of *E. coli* into the thoracic airsac when the birds were 1 week of age. Initial studies demonstrated that mortality was significantly reduced when the challenge culture was mixed with bacteriophage prior to challenging the birds. In subsequent studies, we have shown that an aerosol spray of bacteriophage given to the birds prior to this *E. coli* challenge can prevent the disease, and that an intramuscular injection of bacteriophage provides an effective treatment of this disease. We have demonstrated that bacteriophage can be used to both prevent and treat colibacillosis in poultry and may provide an effective alternative to antibiotic use in animal and human medicine.

Introduction

There will always be a need to find alternatives to antibiotics to both prevent and treat bacterial diseases. The emergence of antibiotic resistance in bacteria of human clinical significance continues to challenge the treatment of these bacterial diseases. There is growing concern that the use of antibiotics in animal production to combat diseases, both at sub-therapeutic levels, as growth promoters, and at therapeutic levels contributes to the emergence of bacteria resistant to antibiotics that have human clinical significance. Although the importance of the use of antibiotics in animal production to the development of antibiotic resistance in bacteria pathogenic to humans is equivocal, the animal industry continues to be pressured to restrict antibiotic use (Bywater 2005). In fact, the European Union banned the use of sub-therapeutic levels of antibiotics in 2006 in all animal production. Therefore, there is a continuing need to find alternatives to antibiotics that has been made more immediate due to government-imposed restrictions on antibiotic use in animal production.

Bacteriophage were co-discovered in the early 1900's by Twort (1915) and d'Herelle (1917). Bacteriophage are viruses that kill bacteria, are ubiquitous in nature, and have no known activity in animal and plant cells. Lytic bacteriophage attach to a target bacterium, inject their genetic material, replicate in the bacteria, and kill the bacteria by lysis, which results in the release of 20 to 200 bacteriophage that can in turn infect additional bacteria. This life cycle offers the advantage of using bacteriophage to specifically target bacterial pathogens without harming beneficial bacteria, and is both self-replicating and self-limiting. With their discovery, the potential of bacteriophage to treat bacterial diseases was immediately recognized. In fact, one of the first applications investigated was work showing bacteriophage efficacy to treat salmonellosis in poultry (d'Herelle 1926). Although bacteriophage therapy of bacterial diseases showed promise, this approach waned with the development of antibiotics. However, there is renewed interest in bacteriophage therapy in a continued effort to reduce the impact of bacterial diseases on human and animal health. The use of bacteriophage to control *E. coli* induced diarrhea in calves, piglets, and lambs was demonstrated by research led by H. W. Smith (Smith and Huggins 1983, Smith et al. 1987). The ability of bacteriophage to treat *E. coli* infections in mice has also been demonstrated (Smith and Huggins 1982). Barrow et al. (1998) demonstrated the ability of bacteriophage to protect chickens from an intramuscular challenge with *E. coli* when the bacteriophage were simultaneously injected at different sites. Soothill (1992) was able to

use bacteriophage to protect mice from infection with *Acinetobacter baumanii* and *Pseudomonas aeruginosa.* The Eliava Institute in Tblisi, Georgia (of the former Soviet Union), has continued bacteriophage research to the present. The Eliava Institute was established in 1923 by Giorgi Eliava a former student of Felix d'Herelle. The Russian research on bacteriophage in human medicine was reviewed by Alisky et al. (1998). Human clinical research is also been carried out in Poland (Ślopek et al. 1981, 1984, 1985, 1987, Weber-Dąbrowska et al. 1987). Biswas et al. (2002) demonstrated that bacteriophage were able to rescue mice from a lethal challenge with a vancomycin-resistant *Enterococcus faecium*. Recently bacteriophage have been shown to have promise in the treatment of urinary tract infections (Nishikawa et al. 2008). There are a number of excellent reviews of bacteriophage therapy (Carlton 1999, Skurnik and Strauch 2006, Hanlon 2007, Parisien et al. 2008).

Colibacillosis is a serious problem in poultry production causing mortality and condemnations (Piercy and West 1976, DeRosa et al. 1992, Barnes et al. 2008). The etiology of this disease is thought to initiate as a respiratory infection known as airsaculitis that can quickly become systemic causing perihepititis and pericarditis and resulting in morbidity and mortality.

We isolated bacteriophage from municipal and poultry processing waste to an isolate of *E. coli* pathogenic to poultry, which is serotype 02, non-motile, and lactose negative (Huff et al. 2002a). We have been engaged in an ongoing effort to determine the efficacy of bacteriophage to both prevent and treat colibacillosis as reviewed in a similar review article (Huff et al. 2005).

Initial Studies

In our initial work we mixed a selected bacteriophage with *E. coli* prior to challenging the birds via a thoracic airsac inoculation and the results of this work are presented in Figure 1. Mortality in the birds challenged only with *E. coli* (10^4 cfu per mL) was 85% (Treatment 2). When 10^4 pfu per mL of bacteriophage was mixed with 10^4 cfu per mL of *E. coli*, mortality was significantly reduced to 35% (Treatment 3), and when 10^8 pfu per mL of bacteriophage was mixed with 10^4 cfu per mL of *E. coli* there was complete protection of the birds (Treatment 4) (Huff et al. 2002a). Although this experimental design is very artificial it did suggest that bacteriophage might be used to prevent colibacillosis and it demonstrated the importance of bacteriophage titer for therapeutic efficacy. In addition, this work provides a relatively simple *in vivo* experimental design to screen bacteriophage for efficacy.

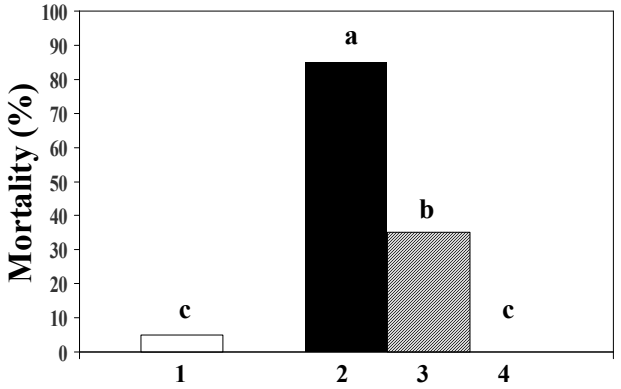

Figure 1. Effect of mixing *E. coli* with bacteriophage prior to challenge. Treatments: 1. Control □, 2. *E. coli* challenge 10^4 cfu ■, 3. *E. coli* 10^4 cfu mixed with 10^4 pfu bacteriophage ▨, 4. *E. coli* 10^4 cfu mixed with 10^8 pfu bacteriophage ▥. Values represent the means of two replicate pens of 10 birds per pen. Values with different letters differ significantly ($P \leq 0.05$) (Huff et al. 2005).

Bacteriophage Disease Prevention Work

The ability of an aerosol spray of bacteriophage to prevent colibacillosis is presented in Figure 2. At 7 days of age the birds were sprayed with bacteriophage and then challenged with *E. coli* either immediately (7 days of age), 1 day (8 days of age), or 3 days (10 days

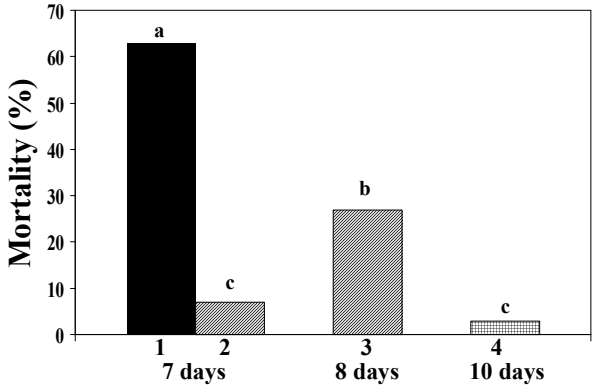

Figure 2. Efficacy of a bacteriophage spray to prevent colibacillosis. Treatment 1 ■, birds challenged with *E. coli* only. Treatments 2 ▨, 3 ▤, and 4 □, birds sprayed with bacteriophage at 7 days of age prior to challenging with *E. coli* at 7, 8, or 10 days of age, respectively. Values represent the mean of four replicate pens of 10 birds per pen. Values with different letters differ significantly ($P \leq 0.05$) (Huff et al. 2005).

of age) after bacteriophage administration. Mortality in the birds only challenged with *E. coli* was 65% (Treatment 1). There was a significant decrease in mortality compared to the control treatment in all the birds administered bacteria prior to being challenged with bacteriophage (Treatments 2, 3, and 4). These data suggest that an aerosol administration of bacteriophage could provide protection of the birds from a consequent challenge with *E. coli* for at least 3 days (Huff et al. 2002b).

Bacteriophage Disease Treatment Work

Studies were conducted to evaluate the ability of bacteriophage to treat colibacillosis when bacteriophage were administered after the birds were challenged with *E. coli*. An aerosol spray administration of bacteriophage was not an effective treatment of colibacillosis (Huff et al. 2003a). However, as can be seen in Figure 3, an intramuscular (i.m.) administration of bacteriophage either immediately (Treatment 2, 7 days of age), 24 hours (Treatment 3, 8 days of age), or 48 hours (Treatment 4, 9 days of age) after the birds were challenged with *E. coli* significantly reduced mortality compared to the birds in Treatment 1, that were challenged with *E. coli* and not treated (Huff et al. 2003a). Additional research has demonstrated that multiple treatments of bacteriophage enhance bacteriophage efficacy to treat colibacillosis in poultry (Huff et al. 2003b). Colibacillosis starts as a respiratory infection that within 48 hours becomes a systemic infection. As can be seen in Figure 4, when bacteriophage were administered as an aerosol spray and then isolated from the blood over a time period of up to 24 hours post administration the titers of bacteriophage were low and only a few birds had any titers at all. However, when bacteriophage were administered i.m., titers of bacteriophage in blood remained above 10^4 pfu per mL in 5 out of 5 birds sampled up to 6 hours post administration. At 24 hours post administration titers were approximately 10^2 pfu per mL, and 4 out of 5 birds sampled had detectable levels of circulating bacteriophage (Huff et al. 2003a). These results demonstrate that once colibacillosis is established in the birds as a systemic infection, treatments with bacteriophage must result in circulating titers of bacteriophage to be effective. We have also demonstrated that i.m. treatment with bacteriophage is comparable to enrofloxacin treatment, and that if enrofloxacin and bacteriophage treatments are combined the treatment efficacy is enhanced (Huff et al. 2004).

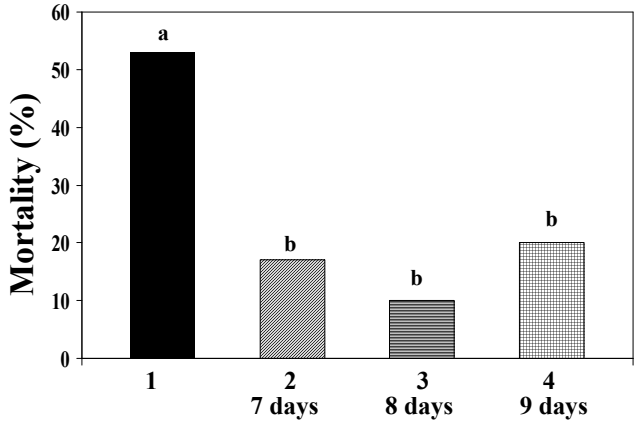

Figure 3. Efficacy of bacteriophage to treat colibacillosis. Treatment 1 ■, birds challenged with *E. coli* and not treated with bacteriophage. Treatments 2 ▨, 3 ▤, and 4 □, birds challenged with *E. coli* and treated with bacteriophage immediately (7 days of age), 24 hours (8 days of age), or 48 hours (9 days of age) after *E. coli* challenge, respectively. Values represent the mean of four replicate pens of 10 birds per pen. Values with different letters differ significantly ($P \leq 0.05$) (Huff et al. 2005).

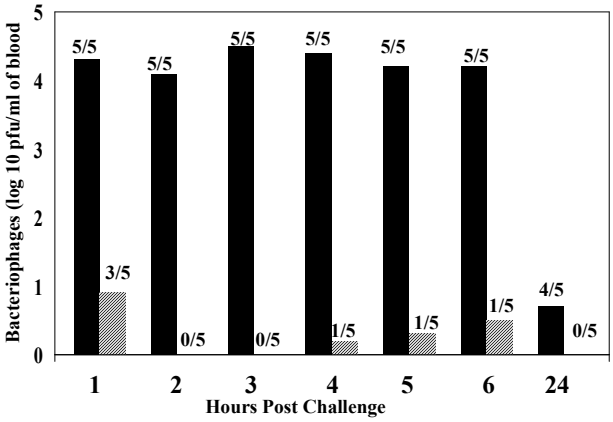

Figure 4. Bacteriophage isolation from blood 1, 2, 3, 4, 5, 6, and 24 hours post challenge with bacteriophage administered i.m. ■ or aerosol spray ▨. Notation above the bars represent the number of birds that were positive for bacteriophage versus the number of birds sampled (Huff et al. 2005).

Conclusions

A common theme of these studies is that if high enough titers of bacteriophage reach the site of infection, bacteriophage will indeed both prevent and treat bacterial diseases. This would seem to be a relatively easy criterion to meet, but it is not. Modern

poultry production facilities contain thousands of birds in each house and multiple houses on a single farm. It would not be practical or economically feasible to administer an i.m. treatment of bacteriophage during an outbreak of colibacillosis in poultry production facilities. These restrictions unique to the efficacy of the use of bacteriophage in poultry production facilities do not preclude the use of bacteriophage in human medicine. Our research clearly suggests that bacteriophage therapy in human medicine has real potential to both prevent and treat human diseases.

We do believe that either *in ovo* injection or treatment in the hatchery with a spray of bacteriophage would prevent the early onset of colibacillosis in poultry, which is thought to be the most critical control point in the etiology of the disease. In addition, an aerosol spray of poultry and litter in production facilities during an outbreak of colibacillosis might prevent horizontal transmission of the disease, as demonstrated in calves (Smith et al. 1987). These applications would provide an inexpensive and effective way to reduce the impact of colibacillosis in poultry, and provide justification to explore the application of bacteriophage therapy to other respiratory diseases of poultry. Bacteriophage treatment of systemic infections in poultry does not appear to be practical unless the animals are extremely valuable, such as breeding stock, or where antibiotic therapy is not available. Although bacteriophage are self replicating, bacteriophage therapy is titer dependent (Huff et al. 2006). Therefore, bacteriophage treatments should be designed to maximize bacteriophage titers.

There has always been a concern with immune interference with long term or repeated bacteriophage treatments. We have demonstrated that bacteriophage treatment efficacy is reduced by approximately 50% when animals are pretreated with the same bacteriophage used for treatment (unpublished data). However, it is possible to circumvent this problem with bacteriophage therapy by using a cocktail of efficacious bacteriophage that differ in antigenicity. Given the diversity and plentiful nature of bacteriophage, it is anticipated that antigentically dissimilar bacteriophage can be found. There is a continuing need to develop *in vitro* tests that will accurately predict *in vivo* therapeutic efficacy of bacteriophage.

Although some promising results of using bacteriophage to reduce food borne enteric pathogens have been documented (Goode et al. 2003, Higgins et al. 2005, Greer 2005, Hagens and Loessner 2007), the log reductions have been modest. There are many reasons why this application of bacteriophage therapy has had limited success such as non-specific binding to digesta and non-targeted bacteria, loss of activity of bacteriophage under the harsh conditions of digestion, and the inaccessible nature of the targeted bacteria, which are often found deep within villus crypts. There is a real need in all animal industries for more research in this area. With a better understanding of the significance of the microbiota of the intestine it may even be possible to use bacteriophage to alter the microbiota to improve the growth of production animals such as sub-therapeutic antibiotics currently do, and this application of bacteriophage has not, but should be pursued.

Bacteriophage can be used to both prevent and treat bacterial diseases and provide an alternative to antibiotic approaches. However, bacteriophage are not a replacement for antibiotics. Applications of bacteriophage need to be targeted with an understanding of where this approach to disease prevention and treatment will not only have efficacy, but will be practical and cost effective.

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