

The effect of bacteriophage treatment to reduce the rapid dissemination of *Salmonella typhimurium* in pigs

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Summary and Implications

Bacteriophage treatment significantly reduced the rapid dissemination of *Salmonella typhimurium* in tonsil and cecum, where the highest number of *Salmonella* was recovered in pigs experimentally infected with *S. typhimurium*. The rapid dissemination of *Salmonella* in market weight pigs prior to slaughter may pose a potential risk in contaminating pork products. Phage treatment should be considered as an intervention strategy to reduce the number of *Salmonella* in pigs.

Introduction

Salmonella spp. in the swine industry causes great concern due to case of human salmonellosis through the ingestion of contaminated pork products. Previous studies reported that the rate of *Salmonella* in market-weight pigs gradually increases from farms, lairage, and ultimately to the slaughterhouse (4, 5). In addition, healthy pigs can become *Salmonella* culture positive within as few as 3 hours after experiment infection as the result of rapid dissemination. The rapid increase of *Salmonella* rates in pigs prior to slaughter should be considered as an important factor to contaminate pork products.

Meanwhile, the reduction of the number of *Salmonella* in pigs might decrease the associated risk of foodborne salmonellosis. Therefore, there has been investigation to develop reduction strategies against *Salmonella* at the preharvest or harvest level. Among the new approaches, *Salmonella*-specific lytic bacteriophage (phage) can be used as a potential method to reduce *Salmonella* in livestock including pigs. The objective of the study was to assess whether phage administration is effective in reduction of *Salmonella* in pigs, focusing on the rapid dissemination of *S. typhimurium*.

Materials and Methods

Bacteria and phage. *S. typhimurium* χ 4232 (nalidixic acid resistant), an isolated strain from clinical pigs, was used for

both challenging pigs and propagation of phage.

Salmonella specific lytic phage (Felix 0-1) was used in the study as a unique phage that is able to attack a broad host range of *Salmonella*. Phage stock was prepared by the semisolid (0.8%) culture method supplemented with CaCl₂ and then filtered (pore size 0.45 μ m).

Enumeration of *Salmonella*. Tissue samples were homogenized with tissue grinder and diluted with phosphate buffered solution. The numbers of bacteria were found by spreading 10-fold serial dilutions on to an XLD agar plate (*Salmonella*-selective media) containing nalidixic acid (50 μ g/ml).

Pigs and Experimental design. Three week-old pigs were purchased from a *Salmonella* spp.-free farm and screened for indigenous *Salmonella* spp. bacteriologically as well as serologically. All pigs were acclimatized for 1 week prior to challenge with *S. typhimurium*. To study rapid dissemination, pigs were sacrificed at 3 hours after challenge with *S. typhimurium* intranasally in concentration of 10⁸, and 10³ colony-forming units (CFU). Tonsil, liver, lung, blood, spleen, ileocecal lymphnode (ICLN), and cecum contents samples were taken at necropsy to evaluate the dissemination of the organism.

The second experiment was done to address the reduction effect of phage administration against *S. typhimurium* in pigs. Pigs were assigned into two groups: untreated group after *Salmonella* infection, and principle (phage treatment) group. Pigs in both groups were intranasally challenged with 5 \times 10⁸ CFU of *S. typhimurium*. Three hours post challenge, pigs in the principle group received 10 ml of 2 \times 10¹⁰ plaque forming units (PFU) of Felix 0-1 phage lysate via oral (6 ml) and intramuscular (4 ml) inoculation. Pigs in the untreated group received *Salmonella* culture lysate that contained sonicated *S. typhimurium*. At 9 hours post infection with *Salmonella* (at 6 hours after phage administration), all pigs in both groups were sacrificed. Tissue samples were taken in the same manner as described above, and then the number of the organisms in tissue samples was counted.

Results and Discussion

Salmonella typhimurium rapidly disseminated in pigs within 3 hours post infection with 10⁸CFU of *S. typhimurium* via an intranasal route (Table 1). This result not only confirmed the previous data(1, 2) of rapid dissemination but also implied that *Salmonella* infection in healthy market weight pigs can occur very quickly during the short periods of both transportation and holding prior to

slaughter. Based on these grounds, any intervention strategy against *Salmonella* at the preharvest level may be helpful to decrease the number of foodborne diseases associated with contaminating pork products. Despite many investigations, intervention strategies have been challenged by the unique properties of *Salmonella*: broad host range, and ubiquitous existence in nature.

Salmonella Felix 0-1 phage lysate treatment reduced the population of *S. typhimurium* in pigs at 3 hours after *Salmonella* infection (Table 2). The significant reduction of *Salmonella* in pigs by phage administration was observed primarily in the tonsil and cecum. Both tissues harbored the highest number of *Salmonella* and are considered as major sources of shedding *Salmonella*, resulting in the spread of *Salmonella* to other pigs.

Although phage treatment didn't eliminate *Salmonella* in pigs, phage administration can decrease the risk of spreading *Salmonella* through the reduction of the number of *Salmonella* in the tonsils and cecum. However, the number of *Salmonella* in cecum contents is still considerably high. Interestingly, these results showed similar patterns to previous studies with phage lysate as a therapeutic tool (3). This could be due to factors that lower phage propagation in gut contents. With further studies combined with other biological means, such as probiotics, the phage effect in the gut could be improved. *S. typhimurium* isolated from pigs treated with phage lysate was not resistant to Felix 0-1 phage. This result suggested that the Felix 0-1 phage is stable in attacking target host

bacteria. Moreover, Felix 0-1 phage may cover almost all *Salmonella* spp. that cause public concerns in livestock, since this phage uses a common and stable component of the *Salmonella* spp. outer membrane as its receptor. This is required for killing host bacteria. We suggest that a phage cocktail containing Felix 0-1 as the principle phage should be considered as an effective short-term intervention strategy against *Salmonella* spp infection in swine.

References

1. DeJong H. and Ekahl M.O. 1965 Salmonellosis in calves: the effect of dose rate and other factors on transmission. *N Z Vet J* 13:59–64.
2. Fedorka-Cray P.J., Kelley L.C., Stabel T.J., Gray J.T. and Laufer J.A. 1995 Alternate routes of invasion may affect pathogenesis of *Salmonella typhimurium* in swine. *Infect Immun* 63:2658–64.
3. Barrow P.A. and Soothill J.S. 1997 Bacteriophage therapy and prophylaxis: rediscovery and renewed assessment of potential. *Trends in Microbiology* 5: 268–271.
4. Morgan I.R., and Krautil F.L., and Graven J. A. 1987 Effect of time in lairage on caecal and carcass *Salmonella* contamination of slaughter pigs. *Epidem Inf* 98:323–330.
5. Williams L.P., and Newell K.W. 1968 Sources of *Salmonella* in market swine. *J Hyg Camb* 66:281–293.

Table 1. Culture positive in tissues 3 hours post challenge with 10^8 , 10^5 CFU of *S. typhimurium* intranasally (n=3).

Challenge dose	Blood	Tonsil	Liver	Spleen	Lung	ICLN	Cecum	Feces
1×10^8 CFU	0/3	2/3	2/3	2/3	0/3	2/3	3/3	1/3
1×10^5 CFU	0/3	3/3	0/3	0/3	0/3	1/3	2/3	0/3

Table 2. The effect of phage lysate treatment against *Salmonella* in pigs challenged with 5×10^8 CFU. Samples were taken after nine hours post challenge with *S. typhimurium* (at 6 hours after phage lysate treatment). The number of *Salmonella* was counted by direct spread method (Number of *Salmonella* per gram of sample).

Groups(n=5)	Blood	Tonsil	Liver	Spleen	Lung	ICLN	Cecum	Feces
Untreated	$<10^2$	9×10^6	$<10^2$	$<10^2$	10^2	3.4×10^3	1.42×10^7	2.2×10^3
Phage treated	$<10^2$	$<10^{2*}$	$<10^2$	$<10^2$	$<10^2$	2×10^2	$3.6 \times 10^{5*}$	$<10^2$

$< \log 10^2$: minimum limit to detect the number of bacteria by direct spread.

* $P < 0.001$