Bacteriophage Therapy in Salmonella Infected Broiler Chickens

B.W.Gannon\textsuperscript{1}, D.E.Halffide\textsuperscript{1}, D.Zammerini\textsuperscript{1}, C.M.Hayes\textsuperscript{1}, V.N.Krylov\textsuperscript{2}, A.Nicolau\textsuperscript{3}, J.Azeredo\textsuperscript{3} and J.M.Roe\textsuperscript{1}

1. Aerobiology, Dept. of Clinical Veterinary Science, University of Bristol, Langford, North Somerset, BS40 5DU, UK
2. Nlgenet. State Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow, 113545 Russia
3. Instituto de Biotecnologia e Bioengenharia, Universidade do Minho, Campus de Gualtar, 4700-057 Braga, Portugal,
   E-mail: ben.gannon@bristol.ac.uk

‘Phagevet-P’ is a multi-centre European Union-funded project evaluating the use of phage therapy as an alternative to antibiotics in poultry production. This ongoing project is targeting salmonella and campylobacter using phage supplied by Russian and Portuguese partners. Preliminary trials on the application of phage therapy against Salmonella Enteritidis-colonised broilers have been successful.

Ninety-six 1-day old broiler chicks were inoculated with 1.5 x 10^5 CFU of S.Enteritidis PT4 S1400/94: kan\textsuperscript{R} by oral gavage in 0.1 ml of PBS. Birds were randomised into 4 groups and housed in sterile isolators. All food, water and bedding were sterilised before use. After 7 days all birds received an oral gavage of 1 ml of 30%w/v CaCO\textsubscript{3} containing either 1.5 x 10\textsuperscript{4} (low dose), 3.0 x 10\textsuperscript{6} (medium dose), 2.9 x 10\textsuperscript{8} (high dose) or 0 PFU (negative control) of phage PVP38. Caecal contents were collected post mortem from six birds from each group at days 1, 2, 4 and 7 post-phage treatment. Salmonella were enumerated from ten-fold serial dilution in SM buffer followed by plating on BGA containing 20\mu g/ml of kanamycin, and on XLD. For phage enumeration the samples were chloroform-treated and plaque assays performed in LB broth containing 0.7% agar, on ten-fold serial dilutions in SM buffer.

There was a statistically significant difference (P<0.05 by T test) in salmonella numbers between the control group and the phage-treated groups throughout the study. The largest difference was at one day post-treatment when the mean salmonella numbers in the high, medium and low phage dose groups were respectively 3.07, 2.37 and 1.34 log\textsubscript{10}CFU/g lower than in the control group. At one day post-treatment there was no statistical difference between the numbers of salmonella in the medium and high dose groups but the numbers in both of these groups were significantly lower than those in the low dose group (P<0.05). There was no significant difference in salmonella numbers between the 3 treatment groups at other time points. The difference between the mean numbers of salmonella in the control group and the mean of the combined treated groups gradually diminished over the seven-day period: 1.62, 0.9 and 0.49 log\textsubscript{10} CFU/g respectively at 2, 4 and 7 days post-treatment.

This study demonstrates that a high dose of phage effects a greater reduction in salmonella numbers than does a low dose, although the effect is short lived. The conclusions of this study concur with those of published studies on phage therapy of campylobacter-colonised broilers, which concluded that to achieve the greatest reduction in pathogen numbers the poultry should be treated at the end of the production cycle.