# Evaluation of the efficacy of bacteriophages-derived lytic enzymes (lysins) to reduce colonization and transmission of *Streptococcus suis* in pigs

Niels Dekker<sup>1</sup>, Annemarie Bouma<sup>2</sup>, Ineke Daemen<sup>3</sup>, Hans Vernooij<sup>3</sup>, Leo van Leengoed<sup>3</sup>, Daniel B. Gilmer<sup>4</sup>, Jonathan E. Schmitz<sup>4</sup>, Vincent A. Fischetti<sup>4</sup>, Arjan Stegeman<sup>3</sup> and Jaap A. Wagenaar<sup>1</sup>

<sup>1</sup>Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Utrecht University, Utrecht, The Netherlands

<sup>2</sup> Ministry of Agriculture, Nature and Food Quality, The Hague, The Netherlands

<sup>3</sup> Faculty of Veterinary Medicine, Department of Farm Animal Health, Utrecht University, Utrecht, The Netherlands

<sup>4</sup> Laboratory of Bacterial Pathogenesis and Immunology, The Rockefeller University, New York, USA

# **OBJECTIVE**

Streptococcus suis causes severe infections in pigs, and occasionally in humans. To control disease in pigs, large amounts of antimicrobials are used. An alternative, more pathogen specific approach could be the therapeutic use of bacteriophage lysins. Our objective was to study the effect of nasal and oral application of lysins  $\Delta$ PlySs1 and PlySs2 [1] on S. suis serotype 9 colonization and transmission, and on clinical signs.

### **MATERIALS AND METHODS**

Two experiments that only differed in lysins doses were performed. Each consisted of one lysins- and one placebo-treated group. In each group 5 pigs were inoculated intranasally with S. suis, and 6 contactpigs were added. Pigs were monitored for two weeks, in which treatment was given to both inoculated (days 3-4 and 8-10) and contact pigs (days 1-4 and 8-10). Per treatment a pig received a combination of  $\Delta$ PlySs1 and PlySs2, in low (0.8 and 0.4 mg) or high doses (1.1 and 3.5 mg) in the two experiments respectively. Saliva and nose swab samples, and tonsillar tissue samples were tested for S. suis by quantitative bacteriological culture.

#### RESULTS

Lysin-treated pigs showed a significant reduction in S. suis loads in saliva (1.27-1.81  $^{10}$ LogCFU) and nose samples (1.67  $^{10}$ LogCFU) on one day (high-dose group) or two days (low-dose group). Transmission rates did not differ between lysin-treated and control groups ( $P_{low-dose}$ =0.530;  $P_{high-dose}$ =0.487), and clinical signs and mortality were comparable.

## **CONCLUSION**

Although phage lysins  $\Delta$ PlySs1 and PlySs2 show a clear lytic activity against S. suis in vitro and strongly reduce S. suis colonization in a mouse model [1,2], they appeared not to be effective in pigs with the current formulation. Application did not reduce S. suis transmission between animals or protect against clinical signs and mortality. Reduction of mucosal colonization was only observed on some days of lysins administration.

## **REFERENCES**

[1] D.B. Gilmer, J.E. Schmitz, C.W. Euler, V.A. Fischetti, Antimicrob Agents Chemother 57(6) (2013) 2743-50.

[2] D.B. Gilmer, J.E. Schmitz, M. Thandar, C.W. Euler, V.A. Fischetti, PLoS ONE 12(1) (2017) e0169180.