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The Live Attenuated *Actinobacillus pleuropneumoniae* Triple-Deletion Mutant Δ*apxIC* Δ*apxIIC* Δ*apxIV-ORF1* Strain, SLW05, Immunizes Pigs against Lethal Challenge with *Haemophilus parasuis*

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***Haemophilus parasuis* and *Actinobacillus pleuropneumoniae* both belong to the family *Pasteurellaceae* and are major respiratory pathogens that cause large economic losses in the pig industry worldwide. We previously constructed an attenuated *A. pleuropneumoniae* serovar 1 live vaccine prototype, SLW05 (Δ*apxIC* Δ*apxIIC* Δ*apxIV-ORF1*), which is able to produce nontoxic but immunogenic ApxIA, ApxIIA, and ApxIVA. This triple-deletion mutant strain was shown to elicit protective immunity against virulent *A. pleuropneumoniae*. In the present study, we investigated whether immunization with SLW05 could also protect against lethal challenge with virulent *H. parasuis* SH0165 (serovar 5) or MD0322 (serovar 4). The SLW05 strain was found to elicit a strong humoral antibody response in pigs and to confer significant protection against challenge with a lethal dose of *H. parasuis* SH0165 or MD0322. IgG subtype analysis revealed that SLW05 induces a bias toward a Th1-type immune response and stimulates interleukin 2 (IL-2) and gamma interferon (IFN-γ) production. Moreover, antisera from SLW05-vaccinated pigs efficiently inhibited both *A. pleuropneumoniae* and *H. parasuis* growth in a whole-blood assay. This is the first report that a live attenuated *A. pleuropneumoniae* vaccine with SLW05 can protect against lethal *H. parasuis* infection, which provides a novel approach for developing an attenuated *H. parasuis* vaccine.**

Actinobacillus pleuropneumoniae is the etiologic agent of porcine pleuropneumonia and has a serious impact upon animal welfare and economics in the pig rearing industry (1). Several factors have been identified that are involved in the pathogenicity of *A. pleuropneumoniae*, of which the pore-forming exotoxins are probably the most important (2). The 15 serovars of *A. pleuropneumoniae* secrete different combinations of four exotoxins (ApxI, ApxII, ApxIII, and ApxIV) belonging to the RTX toxin family (1). ApxI and ApxIII are encoded on classical RTX operons in a *CABD* manner, and the ApxII operon in all *A. pleuropneumoniae* serovars is truncated, having only *CA* genes and missing the secretion genes *BD* (1). The ApxIV gene is not a classical RTX toxin gene and is expressed only in infected pigs (3). There is a correlation between virulence and the pattern of Apx toxin production, and *A. pleuropneumoniae* serovars 1, 5, 9, and 11, which produce ApxI and ApxII, are regarded as the most virulent (4).

Live attenuated vaccines hold much promise because protective antigens are produced in a natural context and because live vaccines have a greater ability to stimulate the production of cytokines, including interleukins, tumor necrosis factor, and interferons, which are known to play an important role as immune modulators (5). Previous studies have confirmed the feasibility of engineering mutant strains of *A. pleuropneumoniae* via the use of selectable antibiotic resistance determinants (6), but such strains are unsuitable as vaccines owing to biosafety concerns (7). We have therefore developed methods for the construction of *A. pleuropneumoniae* vaccine strains that avoid the use of antibiotic resistance genes. We previously developed a double-deletion Δ*apxIC* Δ*apxIIC* mutant strain, SLW03, of *A. pleuropneumoniae* serovar 1. Upon homologous or heterologous challenge, there was no overt clinical disease or mortality in pigs vaccinated with SLW03. These results, combined with the fact that the strain contains no foreign DNA, emphasize the potential of SLW03 as a live attenuated vaccine (8).

However, previous studies showed that ApxIVA retains weak hemolytic activity in the presence of ORF1, a protein encoded immediately upstream of *apxIVA* (3), although it remains to be determined whether ORF1 acts in the same way as the ApxC post-translational activators of other Apx toxins. We therefore constructed a live mutant by introducing an *apxIVA-ORF1* deletion into the double-mutant Δ*apxIC* Δ*apxIIC A. pleuropneumoniae* strain SLW03 (9). This triple-deletion Δ*apxIC* Δ*apxIIC* Δ*apxIV-ORF1* mutant strain, named SLW05, was found to have reduced virulence in both mice and pigs compared to that of SLW03 and could elicit protection against *A. pleuropneumoniae* homologous and heterologous serovar lethal challenge (9).

Haemophilus parasuis is the causative agent of Glässer's disease, and it has become one of the most important bacterial pathogens of livestock worldwide (10). So far, 15 *H. parasuis* serovars have been described, but up to 25% of isolates in some countries could not be allocated to any known serovar (11, 12). Although vaccination is commonly considered to be the most effective way to control and eradicate infectious disease (13), currently, there is

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