

Novel Vaccine Delivery Technology

Philip Willson

Vaccine & Infectious Disease Organization, 120 Veterinary Road, Saskatoon, SK S7N 5E3.

Email: philip.willson@usask.ca

■ Introduction

Infections of the respiratory and GI tracts are among the most common diseases of piglets worldwide and cause substantial economic loss to swine producers. Effective immunity to these infections needs a specific response at the surface of the tract (Bozic *et al.*, 2002). However vaccine formulation and delivery to these surfaces remains a major challenge. Recent evidence of this type of immune response in the intestinal mucosa, using a unique animal model system developed at VIDO, supports the importance of delivering antigen directly to the site (targeting) where an effective immune response is desired (Gerdtts *et al.*, 2001). An alternative mucosal immunization can be achieved by aerosol delivery to the upper respiratory tract and by including immuno stimulatory (CpG) DNA in the vaccine (Alcon *et al.*, 2003).

Although it is more practical to deliver vaccines orally, it is necessary to prevent the vaccine from being digested using, for example, a special coating process (Liao *et al.*, 2003). An oral live vaccine has been shown to protect pigs from ileitis (Kroll *et al.*, 2004) and a corn-based delivery system is also promising for control of TGE (Lamphear *et al.*, 2004).

Another way to use DNA technology to improve vaccines for pigs is by developing adenovirus as a platform for a family of vaccines. The overall approach is to select a common swine virus that causes no disease (porcine adenovirus) and replace unnecessary genes with DNA encoding a vaccine antigen to produce an engineered vaccine. The engineered vaccine acts something like a modified live vaccine (MLV). However, one significant difference is that MLV can revert to virulence, whereas the engineered vaccine is based on a harmless virus so that there is no potential for this to become virulent.

■ **Protective Immunity from Novel Adjuvants, Delivery Systems and Immunization Protocols**

Many studies have demonstrated the beneficial effects of CpG oligonucleotides (ODN), particularly in mice. This is a type of “danger signal” that is common in bacteria and rare in mammals. We tested whether a lipid-based vaccine formulation with CpG-ODN, would protect against pleuropneumonia and limit reaction at the vaccination site. It is important that vaccines for food-producing animals protect against disease while also preserving high meat quality, and limiting the area of reaction in edible tissues is an important consideration.

Increased levels of OmlA-specific antibody were detected in animals immunized with OmlA and CpG-ODN formulated in the delivery system Biophasix™-vaccine targeting adjuvant (VTA), compared to pigs immunized with various controls (Alcon *et al.*, 2003). In addition, the protection induced by VTA/CpG formulation was similar to that induced by the commercial adjuvant VSA; however, VTA formulations caused significantly less tissue damage than VSA. Examination of the tissues revealed that the commercial adjuvant, VSA, caused cell infiltration consisting predominantly of mononuclear cells, and necrosis. In contrast, the degree of inflammation induced by VTA formulations was significantly lower ($P < 0.001$) than those induced by the commercial adjuvant VSA.

■ **Porcine Adenovirus-3 (PAV-3) Vector Based Porcine Vaccines**

Vaccination is an effective way to protect pigs from disease. Since many disease-causing organisms enter at mucosal surfaces (respiratory and gastrointestinal tract), immunity is required there in order to block the initial infection and thus reduce the chances of developing disease.

Production of live vaccines is cost effective. However, the live vaccines produced by conventional means ensure that the live organism is always present in the animals, which can mutate back to a virulent form (in vivo recombination) and cause fatal disease. Thus, new approaches have to be developed for the efficient delivery and the production of safe and cost effective viral vaccines. One way to do this is to develop vector based vaccines. Such attenuated live viral vectors can be engineered to carry genes for other pathogens thus making it possible to immunize animals and get protective immunity at the mucosal surface to several disease organisms at one time.

Our recent objectives have been to make porcine adenovirus expressing vaccine antigens of disease-causing organisms (starting with PRRS) and then test immune response to vaccination and protection from disease (Zakhartchouk *et al.*, 2003).

We tested the safety and immunogenicity of recombinant PAV-3 expressing PRRS proteins in pigs. Five- to six-week old crossbred pigs seronegative for PRRS were randomly divided into groups. All pigs had low serum anti-PAV-3 titers. The pigs were vaccinated twice, 25 days apart with recombinant PAV310, PAV312 or PAV300, either oro-nasally or subcutaneously (5×10^7 IU / ml). At day 40, all pigs were challenged intranasally with PRRS virus (10^6 TCID₅₀ / ml of ATCC VR-2332). Serum samples were collected at different times and analyzed for PRRS specific antibodies using HerdCheck PRRS Virus antibody test kit 2XR (IDEXX Laboratories Inc.) and virus neutralization tests.

Very low antibody levels were found in oro-nasally vaccinated pigs before exposure to PRRS. However, 13 days later the groups immunized with PAV310 or PAV312 had more antibody than the control (PAV300) group. A similar response was seen in animals immunized subcutaneously. Interestingly, PRRS specific IgG titers remained high in pigs immunized subcutaneously, while titers of the oro-nasally vaccinated pigs declined.

At 21 days post challenge, pigs immunized oro-nasally with PAV310 developed a higher PRRS virus neutralizing antibody response compared to the control (PAV300) group. However, pigs vaccinated subcutaneously developed weak PRRS virus neutralizing antibody responses, even after 21 days post challenge.

After challenge, pigs developed mild fever and interstitial pneumonia; however, there was no evidence of more serious disease (proliferation of type 2 pneumocytes or necrosis). VIDO has been awarded one patent and has filed three additional patents on use of PAV-3 as a live virus vector.

■ Practical Needle-Free Vaccine Delivery

Another experiment compared protection of pigs that were vaccinated at 6 and 9 weeks of age with PleuroStar-APP® delivered by Agro-Jet™ or by conventional IM routes and challenged at 10 weeks of age with *Actinobacillus pleuropneumoniae* serotype 1. A secondary objective was to measure the

amount of residual vaccine left on the skin surface after each method of administration.

Healthy, crossbred piglets (8 pigs per group) without previous exposure or vaccination against *A. pleuropneumoniae* were used. The commercial vaccine PleuroStar-APP®, was administered intramuscularly to the IM group using a 1" x 18 ga needle and to the AJ group using Agro-Jet® at a pressure of 220 psi. Pigs were vaccinated at 6 weeks of age (Day 0), and 3 weeks later (Day 21). An absorbant cotton swab was applied to the vaccination site immediately after the vaccine was administered. The amount of residual vaccine remaining on the skin surface was determined as the change in weight of an absorbent cotton swab.

All pigs were housed together in a pen and challenged, 5 or 6 at a time, by exposure to *A. pleuropneumoniae* in a plexiglass chamber. Whether they were challenged first or last had no effect on the results ($p > 0.8$).

In summary of the results, there were no post-vaccination reactions in the pigs given the commercial vaccine by either technique that were attributable to the vaccination process. Significantly more vaccine material remained on the skin after vaccination using Agro-Jet® compared to needle injection; however the vaccine was administered by staff with more training and experience with the conventional needle and syringe method. All immunized pigs had an immune response to both antigens that were tested and developed a significant antibody titre after the second immunization. The method of vaccination resulted in no difference ($P > 0.05$) in the response, although there was a tendency for pigs immunized by the Agro-Jet® to have higher IgG2 titers to both antigens and to have better survival, lower mortality and fewer sick days. The Agro-Jet® treatment group had significantly ($p < 0.05$) less disease (lower clinical score after challenge). There were no observable differences at the postmortem examination, but the pigs immunized using the Agro-Jet® tended to have less pneumonia and a lower rate of infection. Hence, pigs immunized using the Agro-Jet® developed protective immunity that was as good as, and arguably better, than was developed by pigs immunized using a conventional needle and syringe.

■ Conclusions

Strong respiratory and GI tract immunity is essential for swine health. New knowledge, strategies and tools are being developed to reach the goal of improved protection from respiratory disease. Improved technologies to deliver

vaccines to the target location are important for both killed and live vaccines, and most of these are still in development. Experimentally, CpG has been shown to enhance immunity from killed vaccines. As an alternative, a new generation of live vaccines based on using a harmless swine virus as the vector is in development. A new challenge for swine producers will be to master the logistical problems associated with needle free delivery of vaccines for improved immunity and efficient production.

Visit our website for current details of VIDO research projects and get "*Vaccination Guidelines For Swine: How to get the maximum benefit when vaccinating your swine herd*" from:

<http://www.vido.org/producers/techgroups/swine/publications.php#Vaccination>

■ Funding Sources

VIDO acknowledges the support of Ontario Pork, Manitoba Pork Council, Sask Pork, Alberta Pork, Alberta Agricultural Research Institute, Saskatchewan Agriculture Development Fund, Saskatchewan Health Research Foundation (SHRF), Canadian Network for Bacterial Pathogens of Swine, NSERC, and commercial partners.

■ References

- Alcon V, Vega-Lopez MA, Willson P, Gomis S, Hecker R, Foldvari M, Baca-Estrada ME. Combination of CpG-DNA and Biophasix™ -Vaccine Targeting Adjuvant (VTA-2) Promotes strong systemic and mucosal immune responses after subcutaneous and intranasal immunization in a swine model. 11th International Congress of Mucosal Immunology, June 16-20. 2002 Orlando, FL. 1640.
- Alcon VL, Foldvari M, Snider M, Willson P, Gomis S, Hecker R, Babiuk LA, Baca-Estrada ME. Induction of protective immunity in pigs after immunisation with CpG oligodeoxynucleotides formulated in a lipid-based delivery system (Biphaxis). *Vaccine*. 2003 May 16;21(17-18):1811-4.
- Bozic F, Lackovic G, Stokes CR, Valpotic I. Recruitment of intestinal CD45RA+ and CD45RC+ cells induced by a candidate oral vaccine against porcine post-weaning colibacillosis. *Vet Immunol Immunopathol*. 2002 Jul;86(3-4):137-46.

- Gerds V, Uwiera RR, Mutwiri GK, Wilson DJ, Bowersock T, Kidane A, Babiuk LA, Griebel PJ. Multiple intestinal 'loops' provide an in vivo model to analyse multiple mucosal immune responses. *J Immunol Methods*. 2001 Oct 1;256(1-2):19-33.
- Kroll JJ, Roof MB, McOrist S. Evaluation of protective immunity in pigs following oral administration of an avirulent live vaccine of *Lawsonia intracellularis*. *Am J Vet Res*. 2004 May;65(5):559-65.
- Lamphear BJ, Jilka JM, Kesl L, Welter M, Howard JA, Streatfield SJ. A corn-based delivery system for animal vaccines: an oral transmissible gastroenteritis virus vaccine boosts lactogenic immunity in swine. *Vaccine*. 2004 Jun 23;22(19):2420-4.
- Liao CW, Chiou HY, Yeh KS, Chen JR, Weng CN. Oral immunization using formalin-inactivated *Actinobacillus pleuropneumoniae* antigens entrapped in microspheres with aqueous dispersion polymers prepared using a co-spray drying process. *Prev Vet Med*. 2003 Sep 30;61(1):1-15.
- Zakhartchouk A, Zhou Y, Tikoo SK. A recombinant E1-deleted porcine adenovirus-3 as an expression vector. *Virology*. 2003 Sep 1;313(2):377-86.