

New Applications for Health and Innovation in Veterinary Medicine

Lorne A. Babiuk

Vaccine & Infectious Disease Organization, University of Saskatchewan, 120 Veterinary Road, Saskatoon, SK S7N 5E3; **Email:** lorne.babiuk@usask.ca

■ Introduction

Throughout the history of livestock production, infectious diseases have been a problem. Indeed, one of the greatest impediments to the economics of livestock production today is the losses due to infectious diseases. Regardless of the advances made in feed conversion efficiency, nutrition and genomics, if only one animal dies, all the other animals must be that much more efficient to meet the production goals of the producer.

In addition to the endemic diseases that plague livestock production, the emergence of new diseases or re-emergence of diseases to which populations are totally susceptible are especially worrisome. For example, the emergence of Nipah virus in Malaysia led to the destruction of millions of pigs (Chua et al., 1999). It is predicted that similar epidemics will occur in the future. In the last 30 years, there have been approximately 30 newly emerged or re-emerged diseases, many of which can infect multiple species (Morens et al., 2004). Secondly, introduction of rare, but devastating diseases into intensive livestock production regions or facilities, via bioterrorism and agro-terrorism, could have devastating consequences to the industry. Some examples of economic losses due to introduction of disease to regions or countries are well illustrated in the case of bovine spongiform encephalopathies (BSE) in the United Kingdom and Canada in the last decade. In both of these instances, the direct cost of compensation to producers was in the billions of dollars. In 2001, foot-and-mouth disease introduction into the United Kingdom cost the industry between \$6-30 billion. Similarly, avian influenza introduction into poultry flocks occurs on a relatively regular basis (Reynolds, 2005). However, the greatest concern about novel avian influenza viruses is their potential to produce human pandemics. For example, there were over 17 million birds killed in British Columbia during 2005 as a result of detection of a novel avian influenza virus in birds. These examples demonstrate the need for both rapid detection methods to limit the

spread of infectious diseases as well as the need to develop novel control measures to limit the economic costs associated with such infections. For example, a modelling study in California calculated that if foot-and-mouth disease was detected within two weeks of introduction into an area of California, then 81% of the herds would be saved. Delaying the diagnosis by a single week would result in only 18% of herds being spared. This model also calculated that the costs of containing the disease in one county (Tulare) was \$3 billion vs. a \$9 billion if it spread to other counties in California (Ekboir, 1999).

These statistics clearly demonstrate the need for novel, more effective control and treatment methods to ensure the viability of the livestock industry. The following report will describe some of the recent advances in disease control, which are being applied to improving efficiency of livestock production. This will be achieved by advances in our understanding of the pathogenesis of many infectious agents of livestock, the genomic sequencing of many of the virulence genes of infectious agents, combined with advances in immunology, genomics and host resistance to infection. These advances place us in an era of unprecedented opportunities to better understand the disease processes (pathogenesis) and, therefore, develop safer and more effective control methods for all pathogens. The current review will focus on methods that are currently being used in veterinary medicine and in swine production, as well as describe futuristic methods to reduce diseases using novel immune modulators and vaccines.

■ Control of Infectious Diseases

Currently, three methods are available to control infectious diseases:

- antimicrobials
- vaccines
- immunomodulators.

Antimicrobials have been one of the most attractive and misused methods of treating swine suffering from bacterial diseases. Some of these antibiotics have also been used prophylactically. A similar approach has not been successful for viral infections due to the lack of effective antiviral agents for many common viral diseases of animals. Unfortunately, the over-use or inappropriate use of antibiotics has resulted in the rapid evolution of bacteria that are resistant to the commonly available cheap antibiotics. As a result, there has been extensive pressure on the livestock industry to reduce the use of antibiotics, especially as prophylactics (Miller et al., 2005). Indeed, a number of countries have banned prophylactic use of antibiotics (Casewell et al., 2003). Unfortunately, this has not necessarily reduced the actual tonnage

of antibiotics used to control infections, since larger amounts are now being used as therapeutics. As a result, we need to rely more on alternative approaches to disease control. These include vaccines and immunomodulators. In the case of vaccines, these products need to be directed against a specific pathogen, not broad-spectrum control as in the case of antibiotics. For the specific diseases where vaccination has been developed, this approach has proved to be very successful in reducing the economic losses due to infectious diseases (Pastoret et al., 1997). Vaccination is the most cost-effective approach for the management of infectious diseases. However, for this approach to be successful, animals must be immunized prior to exposure. Thus, vaccines are really only effective if they are used prophylactically (reviewed in Babiuk, 2002).

■ Vaccines

The underlying principle behind vaccination is that exposure to a foreign agent results in stimulation of immunity to the various components that are present in the vaccine. Recognition of these components by the immune system results in expansion of the lymphocytes that specifically recognize the antigens and produce antibody or cell-mediated immunity. Upon subsequent exposure to the pathogen, a number of lymphocytes that can rapidly respond and develop antibody or cell-mediated immunity is shortened, such that animals will clear the pathogen before clinical signs appear.

The majority of licensed vaccines used in veterinary medicine and in swine operations are produced by conventional methods using principles similar to those initially developed by Jenner and Pasteur 200 and 100 years ago, respectively. Although these vaccines were developed empirically with very little knowledge of the specific antigens involved in inducing immunity or the specific immune responses required for inducing protection, in many instances they have been able to limit the degree of economic losses caused by various infectious diseases.

A newer type of vaccine has been developed by building on the principle of immunological memory. Because not all of the components of a pathogen are required for inducing protection of immunity, researchers have used recombinant DNA technology to identify and clone the one or two protective components that are required in a vaccine. These components were introduced by recombinant DNA technology and used as effective vaccines (Gerlach et al., 1992). One example which demonstrates this technology is in *Actinobacillus pleuropneumoniae*. In this case, there are approximately 13 different serotypes of *Actinobacillus pleuropneumoniae* which can cause various degrees of infection in pigs. To protect against all of the different serotypes, it would require 13 different vaccines. Fortunately, using molecular

biology and genomic and immunologic technologies, it is possible to identify individual protective components that cross-react between a number of different serotypes. Two different companies (Novartis and Intervet) have used this approach to register a recombinant vaccine that contains approximately four different components, which provide some degree of cross-protection against all serotypes. Thus, with only four individual components (proteins), it is possible to protect pigs against all 13 serotypes. **Table 1** summarizes the specific antigens and cross-reactivity of these antigens for different serotypes.

Table 1. Antigen cross-reacting in *A. pleuropneumoniae**

	Serotype												
	1	2	3	4	5a	5b	6	7	8	9	10	11	12
Intervet's Porcilis APP													
APX I	X				X	X				X	X	X	
APX II	X	X		X	X	X	X	X	X	X		X	X
APX III		X	X	X			X		X			X	
OMP (42 kD)*	X	X	X	X	X	X	X	X	X	X	X	X	X
Novartis's PleuroStar APP													
APX II	X	X		X	X	X	X	X	X	X		X	X
TbpB	X	X	X	X	X	X	X	X	X	X	X	X	X
CysI	X	X	X	X	X	X	X	X	X	X	X	X	X
OmlA (1)	X	X							X	X		X	X
OmlA (2)					X	X					X		

* X represents cross-reaction of the specific antigen in a specific antigen

A third approach to reducing economic losses from infectious diseases is the use of immune modulators. Recently, it has been shown that all mammals have unique innate immune responses that are mobilized rapidly to any infectious disease insult. Capitalizing on this discovery, it has been possible to identify a number of unique stimulators of innate immunity, which dramatically increases the natural resistance of animals to infectious diseases. Furthermore, it is possible to combine these stimulators of innate immunity with vaccines to get early protection, while specific immunity is developing to a vaccine and to enhance the efficacy of the vaccine.

■ Immune Modulation

A brief summary of how innate immunity is stimulated within minutes or hours following detection of a specific infection is illustrated in **Figure 1**. Detection of infection or “danger” is achieved through a series of novel receptors on mammalian cells. These receptors recognize unique structures on pathogens which are not present in the host. Triggering of these receptors is rapid, resulting in the synthesis and secretion of a family of molecules called cytokines or chemokines. These molecules both attract immune cells to the site of infection, resulting in the killing of the pathogen, as well as creating a cytokine micro-environment which allows the expansion of T and B cells involved in mediating immunity. Furthermore, the constellation of cytokines and chemokines in the environment of the immune cells (T and B cells) not only determines the magnitude of the immune response, but also the quality of the immune response by either favouring a cellular or a humoral response. Thus, we can combine these novel stimulators of innate immunity with vaccines to enhance both the magnitude of the response, as well as to modulate the quality of the response.

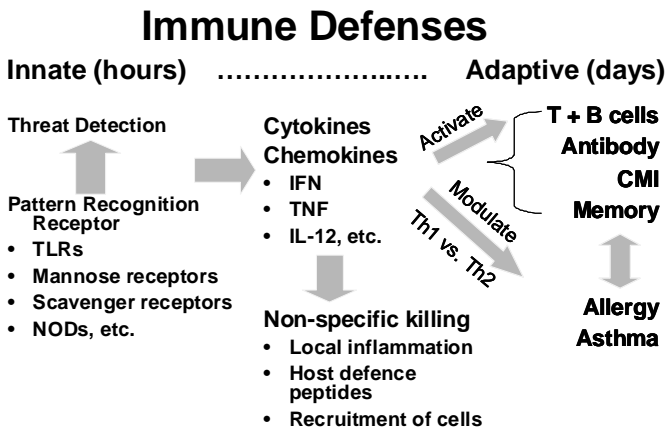


Figure 1. Linkage of innate immune response to adaptive immunity.

As stated above, immune stimulators can be used either as a direct therapeutic/prophylactic agent or as an adjunct to improve vaccine efficacy. Two examples of immune stimulators will be presented. The first immune stimulant to be described is CpG. CpG is a component of all DNA. However, bacterial DNA has more CpG motifs than mammalian DNA and, more importantly, the cytosine residues in bacterial DNA are not methylated, whereas they are in mammalian DNA. Therefore, the mammalian system

recognizes these sequences as novel and potential signatures of a bacterial infection (Krieg, 2000). Therefore, the mammal responds in an aggressive fashion to these CpG motifs, since it believes it is being invaded by an infectious agent. It is possible to synthesize short oligonucleotide sequences containing repeated CpG motifs that can act as danger triggers in most mammals. We have identified unique CpG motifs that are extremely active in stimulating the immune system of most livestock species. Using an *E. coli* model in chickens, we have found that treatment of chickens with CpG dramatically reduces mortality (**Figure 2**). Furthermore, we demonstrated that the animals are resistant to infection for approximately 7 days after treatment. Since animals can be retreated with no adverse effects, it was possible to expand the length of time that animals can be protected as a result of stimulating their immune responses. The efficacy of CpG in preventing infection has been shown to occur in a variety of bacteria, viruses, and parasitic infections (Krieg et al., 2000). Thus, this approach appears to have very broad activity against a wide variety of infectious diseases. Another advantage of CpG is that they are not directed against the infectious agent, but act by activating the host's immune response. This mode of action reduces the likelihood of the infectious agent developing resistance, as occurs with antibiotics. Indeed, the host has evolved this system of defence over thousands of years, thereby providing a level of confidence that resistance will not develop.

Effect of CpG on *E. coli* Induced Mortality in Chickens (SQ) 50 ug

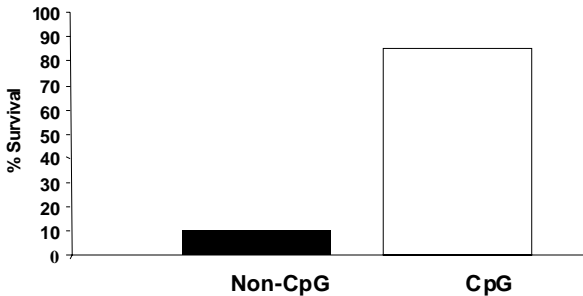


Figure 2. Animals treated with CpG or with a non-CpG ODN 24 hours before infection with *E. coli*. Mortality was measured over a 10-day period.

In addition to acting directly on the host to help clear the pathogen, if CpG is formulated with a vaccine, the efficacy of the vaccine is dramatically improved. Using a purified glycoprotein in cattle, we were able to show that both the quantity and quality of the immune response was improved (**Figure 3**). More

importantly, this enhancement of immunity was reflected in the improved outcome following challenge with a virulent pathogen (**Figure 4**).

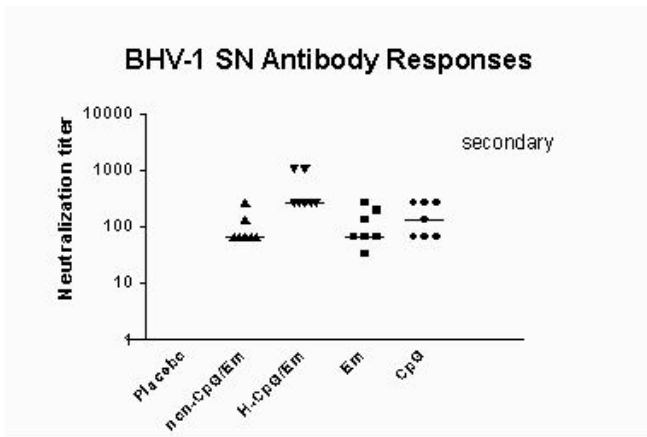


Figure 3. Immune responses of cattle immunized with a glycoprotein gD vaccine. Vaccines were formulated either in Emulsigen; Emulsigen plus CpG-ODN; Emulsigen plus non-CpG ODN or CpG alone. Serum antibody titers were determined following vaccination.

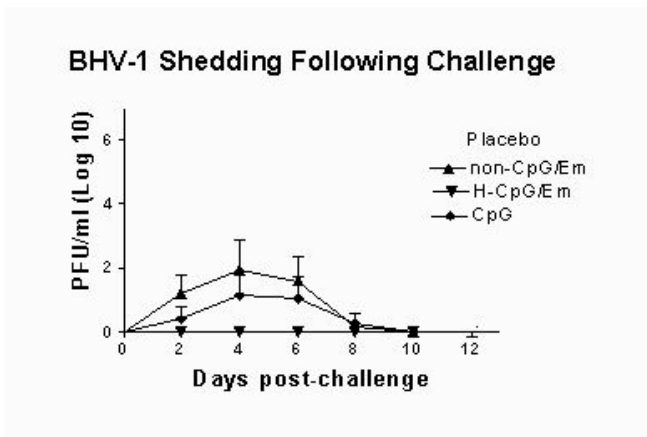


Figure 4. Virus shedding following challenge of cattle with bovine herpesvirus-1. Animals immunized as in Figure 3 were challenged with an aerosol of BHV-1 and monitored for virus shedding from nasal passages.

Similar results were seen in pigs where the level of protection was dramatically improved when CpG was incorporated into the vaccine (Figure 5). This was evident both with respect to the mortality and lung pathology in the animals.

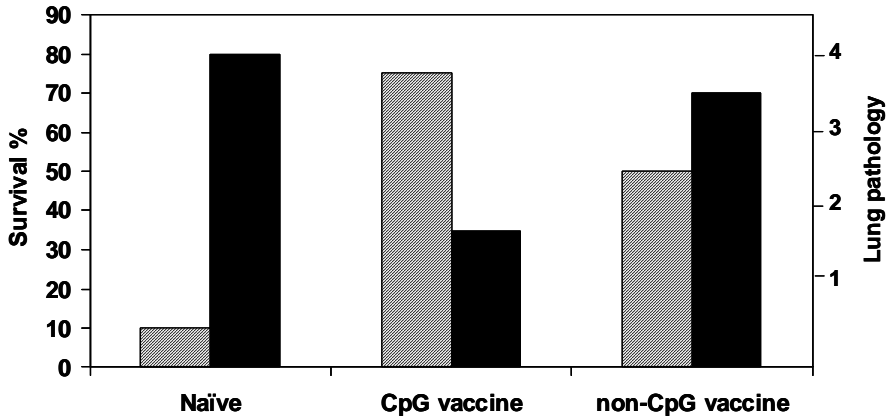


Figure 5. Effect of formulation on protective capacity of the vaccine in pigs. OMLA from *Actinobacillus* was formulated with CpG twice and then animals were challenged with an aerosol of *A. pleuropneumoniae*. Mortality and lung lesions were recorded.

A second family of immunostimulants that are gaining interest are the naturally-occurring host defence peptides. Currently, there are large numbers of these natural peptides produced in the body and they are key mediators of natural immunity. Although many of these host defence peptides can be directly antibacterial, they have also been shown to activate the innate immune system (Finlay and Hancock, 2004). As a result of these observations, it is possible to synthesize whole peptides or portions of these peptides to use as immunomodulators. By synthesizing only portions of these peptides, the cost of such therapeutics should be dramatically reduced. A Canadian company (Inimex Veterinary Research Inc.) is currently exploring these peptides as potential modulators of disease in swine.

■ Formulation

Although it is possible to identify, using genomic and molecular biology approaches, the important protective antigens and to produce these in large quantities, this does not necessarily make the vaccine effective. Regardless of the quality of the antigen used in the vaccine or the presence of immune modulators, if they are not formulated and delivered properly, these vaccines

will not achieve their full potential. Thus, novel formulations and delivery technologies will need to be developed to maximize the benefits of the genomic revolution that has occurred over the last decade. Furthermore, if we could design delivery methods that replace needles, this would reduce tissue damage at the sites of injection and, more importantly, may influence the quality of the immune response. If we can deliver vaccines through mucosal surfaces where mucosal immunity is developed, this would dramatically reduce both the rate of infection, since most pathogens enter via the respiratory tract, and reduce transmission of the agent to in-contact animals. Significant progress has been made at formulating vaccines, which should be able to achieve this goal (**Figure 6**).

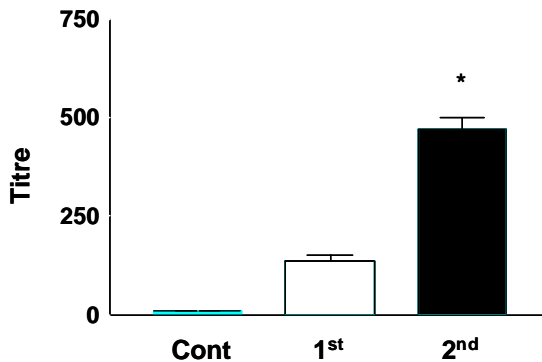


Figure 6. Responses in pigs following intranasal delivery. OMLA antigen from *A. pleuropneumoniae* was incorporated into lipid-based delivery systems and delivered intranasally at Day 0 and boosted 21 days later.

One novel formulation, using polyphosphazenes, has not only been shown to dramatically increase the immune response to vaccines as well as alter the quality of the immune response, but it has been formulated into microspheres to deliver the vaccine intranasally (**Figure 7**). If this type of formulation can be adapted to the pig industry, this could dramatically improve livestock production.

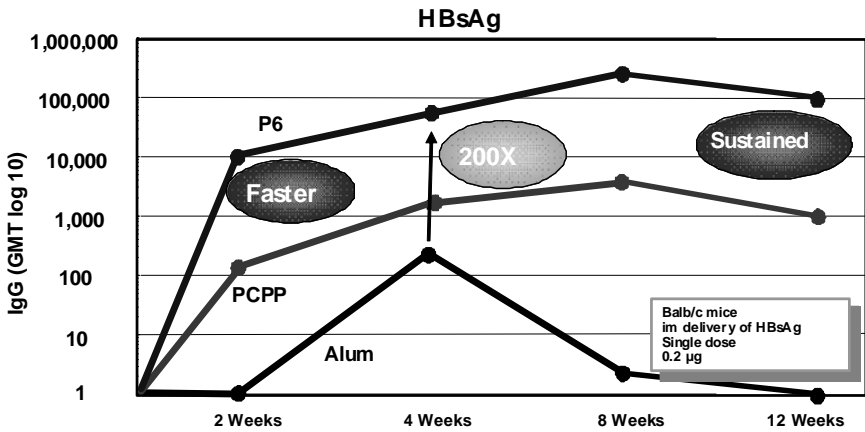


Figure 7. Enhancing Immune Responses by Formulation. Animals were immunized once with either a vaccine in the commercial adjuvant – alum or reformulated with different derivatives of polyphosphazenes.

■ Conclusion

Recent advances in our understanding of the interactions between disease-causing organisms and the host, combined with techniques to identify protective antigens are providing novel approaches to developing more effective vaccines. Furthermore, by combining methods to stimulate the hosts own defenses (innate immune responses), we can induce early protection from a broad-spectrum of infections, in the case of accidental introduction of a disease. These approaches should reduce animal suffering and add significant economic benefits to the producers, as well as improve food safety for the consumer.

■ References

- Babiuk, L.A. 2002. Vaccination: A management tool in veterinary medicine. *The Veterinary Journal*. 164: 188-201.
- Casewell, M., Friis, C., Marco, E., McMullin, P., Phillips, I. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J. Antimicrob. Chemother.* 52: 159-161.
- Chua, K.B., Goh, K.J., Wong, K.T., Kamarulzaman, A., Tan, P.S., Ksiazek, T.G., Zaki, S.R., Paul, G., Lam, S.K., Tan, C.T. 1999. Fatal

- encephalitis due to Nipah virus among pig farmers in Malaysia. *Lancet*. 354: 1257-1259.
- Ekboir, J.M. 1999. The role of the public sector in the development and implementation of animal health policies. *Prev Vet Med*. 40: 101-115.
- Finlay, B.B. and Hancock, R.F. 2004. Can innate immunity be enhanced to treat microbial infection. *Nat Rev Microbiol*. 6: 497-504.
- Gerlach, G.F., Anderson, C., Potter, A.A., Klashinsky, S., and Willson, P. 1992. Cloning and expression of a transferrin-binding protein from *Actinobacillus pleuropneumoniae*. *Infection and Immunity* 60: 892-898.
- Krieg, A.M. 2000. The role of CpG motifs in innate immunity. *Curr. Opin. Immunology*. 12: 35-43.
- Krieg, A.M., Hartman, G., and Yi, A.K. 2000. Mechanism of action of CpG DNA. *Curr. Topics in Microbiology and Immunology*. 247: 1-21.
- Miller, G.Y., McNamara, P.E., Singer, R.S. 2005. Stakeholder position paper: Economist's perspectives on antibiotic use in animals. *Prev. Vet. Med.* In press.
- Morens, D. M., Folkers, G. K., Fauci, A. S. 2004. The challenge of emerging and re-emerging infectious diseases. *Nature* 430: 242-249.
- Pastoret, P-P., Blancou, J., Vannier, P., and Verschuerrren, C. 1997. In: *Veterinary Vaccinology*, Elsevier. Amsterdam. pp. 853.
- Reynolds. D. 2005. Avian Influenza. *Vet Rec*. 157: 598.