Vaccinology for Bovine Practitioners: Immunization for Bacterial Respiratory Tract Infections

D. Scott McVey and **M.M. Chengappa**[2]
[1]*Biodevelopment Department*
Rhone Merieux, Inc.
115 Transtech Drive
Athens, GA 30601
[2]*Department of Diagnostic Medicine / Pathobiology*
College of Veterinary Medicine
Kansas State University
Manhattan, KS 66506

Veterinarians have vaccinated calves for many years against bacterial pathogens. Many diseases have been successfully controlled by use of bacterins or toxoids in adjuvants. Brucellosis and clostridial toxemias are good examples of diseases that have been controlled in part by vaccination. However, some diseases are still prevalent despite widespread use of many types of vaccines. Pneumonic pasteurellosis of cattle and associated bacterins and toxoids are classic examples of this problem. Bronchopneumonia caused by *Pasteurella* (*P.*) *haemolytica* and *P. multocida* still account for huge losses in the dairy, stocker and feedlot industries (Babiuk *et al.*, 1987).

It is interesting to note that the research and development community at various organizations has been very active for may years in bovine respiratory disease research. Numerous virulence factors have been described and characterized. The pathogenic mechanisms of pasteurellaceae have been defined more completely (reviewed by Babiuk and Potter, 1994; Gonzalez and Maheswaran, 1993). In short, practicing veterinarians and research scientists know a lot about these bacteria and the diseases they cause. Unfortunately, this has not yet been completely integrated into practical vaccinology.

There are several reasons why some vaccination strategies have not worked. Most bacterial antigens are extremely complex. For example, heat shock stress of *Salmonella typhimurium* or *Escherichia coli* induces synthesis of numerous protein antigens (Murray and Young, 1992). Many of these antigens are expressed when the bacteria are in phagocytes. The pili, flagella, outer membrane proteins, endotoxin, cell wall-associated structures, capsular polysaccharides, and multiple toxins may all be important in the pathogenesis of infection. Specific, adaptive immunity to all of these components may be important and contribute to a state of immunity (Salyers and Whitt, 1994).

Another level of complex antigenicity is expressed when many pathogenic bacteria survive in multiple environments in the host species. For instance, in healthy, non-stressed cattle, *P. haemolytica* serotype 2 is part of the upper respiratory flora (Magwood *et al.*, 1969). In this situation, this bacterium exists in harmony with many other normal flora. With stress, and probably other poorly-defined factors, *P. haemolytica* serotype 1, biotype A (PhA1) increases in number and colonizes the entire upper respiratory tract and tonsils of cattle. This overgrowth of PhA1 precedes bronchial pneumonia (Frank and Smith, 1983). As the bacterial bronchopneumonia progresses, the bacteria must survive in a phagocyte-rich environment of low 0_2 tension and low pH. These metabolic stressors alter the antigenic nature of the PhA1. Expression of adhesins, iron-assimilation proteins and agglutinating antigens further complicate the definition of a composite set of protective antigens (Gonzalez and Maheswaran, 1993).

At present, production technologies do not insure that all or that even most of the protective antigens will be produced during cultivation of the *P. haemolytica* (Gatewood *et al.*, 1994; Mosier *et al.*, 1994). Some newer vaccines for PhA1 do contain some of the important antigens such as leukotoxin and somatic components. Further work is needed to define a broad set of protective antigens. It is not clear that experimental challenge studies alone can completely determine necessary antigen combinations for improvement of vaccines. Studies of vaccine effectiveness in the field and molecular defi-

nition of pathogenic mechanisms and immune effector mechanisms will be very important in this process.

Another problem that compromises the field effectiveness of all bovine respiratory disease complex vaccines is the historical, market-driven timing of immunization (Ribble *et al.*, 1995). Many calves are vaccinated at the time of assembly or upon arrival to stocker or feedlot units. Therefore, immunization occurs at the same time as, or after, exposure to many pathogenic organisms. Further, response to PhA1 vaccine antigens is compromised by physiologic stressors of shipping and immunologic stress of concurrent viral infections. Many animals have a net reduction in circulating antibody at these times (McVey, 1987).

It is evident that several changes in our immunization programs must occur in order to see real improvement in vaccine effectiveness. First production technologies must adapt methods to produce appropriate antigens. Second, we must employ vaccine formulation strategies and delivery systems to improve immune responses. Third, criteria for field effectiveness studies must be better defined.

One means of delivering antigens to the mucosal surfaces of the intestine is the microencapsulation of antigens (Bowersock *et al.*, 1994; reviewed in Wilding *et al.*, 1994). This type of immunization has the potential advantage of stimulating secretory antibody (IgA) responses that could help protect at the upper respiratory tract surface (Liebler *et al.*, 1988; Parsons *et al.*, 1989). This could be particularly valuable to block bacterial adhesins. Microencapsulated antigen mixtures could be given orally in delayed-release preparations to stimulate tonsillar, Peyer's patch and other mucosal-associated lymphoid tissues (MALT).

The most common strategy for the immunization of animals has been parenteral injection of antigen to induce a primary immune response followed in 2 to 4 weeks with a booster dose of the antigens (Srinand *et al.*, 1995). This approach has worked and continues to work well for many antigens. There are some important factors that must be considered to determine the formulation and timing strategies in the gut (Wilding *et al.*, 1994). First, the mucosal-associated lymphoid tissue (MALT) is constantly exposed to numerous antigens introduced via biologic processes of eating, breathing and copulation. Part of this continual exposure is the microbial mass of the normal flora. Many antigens of the enterobacteriaceae and pasteurellaceae are shared. Therefore, the appropriate protective antigens (or antigen sets) must be selected and then presented frequently in relatively high concentrations. This process could be potentially cost-limiting. A possible solution to this problem is to use linker molecules to target antigens to antigen-processing cells of the MALT in a delayed-release formulation (Bodmeier *et al.*, 1989). These technologies are currently being developed. Such vaccines might be delivered as a capsule in bolus form or they might be suspended in a delivery solution. Either process would require handling cattle but could be successful. If appropriate targeting strategies and antigen stabilizing methodologies can be developed, oral immunization through feed or water could be accomplished.

Below are two examples of how such strategies could be used (Tables 1 and 2). With time and continued development, broad sets of antigens could be included in these oral immunogens.

Table 1. Cow-calf/Dairy

1. Fall	Immunize pregnant cows by mixing antigens in pelleted feed, mixed feed, or supplement mixtures-continuously for 4-6 weeks.
2. Spring	Introduce antigen mixtures via salt supplements or other highly palatable feedstuffs after calves are 2 months of age.
3. Weaning	Reuse spring, suckling-calf mixture with balance of antigens towards upper respiratory pathogens; then:

Table 2. Feedlot Cattle

1. Arrival	Continue product as in weaning rations, drench or bolus if necessary, use for 45 days.
2. Finishing	Gradually reduce dose over 3 week period to 70 days.

Changing these types of immunization protocols depends on the adaption of industrial fermentation, processing and testing procedures. However, like any immunization program, ultimate success depends not on serology or efficacy data, but on field effectiveness. Oral immunization on a broad scale has been very successful against many diseases and recently against rabies (Brochier *et al.*, 1990). Oral immunization can be very effective.

References

1. Babiuk, LA; Lawman, MJP; Biol, MI; *et al.* (1987). Bovine respiratory disease: pathogenesis and control by interferon In *A Seminar in Bovine Immunology.* Western States Veterinary Conference. pp. 12-24, Veterinary Learning Systems Co., Inc. 2. Babiuk, LA; and Potter, AA. (1984). Veterinary vaccines. *Biotech. Adv.* 12: 489-523. 3.Bodmeier, R; Chen, H; and Paeratakul, O. (1989). A novel approach to the oral delivery of micro- or nanoparticles. *Pharm. Res.* 6: 413-417. 4. Bowersock, TL; Shalaby, WSW; Levy, M; *et al.* (1994). Evaluation of an orally administered vaccine, using hydrogels containing bacterial exotoxins of *Pasteurella haemolytica*, in cattle. *Am. J. Vet. Res.* 55: 502-509. 5. Brochier, B; Thomas, I; Baudin, B; *et al.* (1990). Use of vaccinia-rabies recombinant virus for the oral vaccination of foxes against rabies. *Vaccine* 8: 101-104. 6. Frank, GH. (1979). *Pasteurella haemolytica* and respiratory disease in cattle. *Proc. U.S. Animal Health Ass.* 83:153-160. 7. Frank, GA; and Smith, PC. (1983). Prevalance of *Pasteurella haemotytica* in transported calves. *Am. J. Vet. Res.* 44:981-985. 8. Gatewood, DM; Fenwick, BW; and Chengappa, MM (1994). Growth-condition dependent expression of

Pasteurella haemolytica Al outer membrane proteins, capsule, and leukotoxin. *Vet. Microbiol.* 41:221-233. 9. Gonzalez, CT; and Maheswaran, SK. (1993). The role of induced virulence factors produced by *Pasteurella haemolytica* by *Pasteurella haemolytica* in the pathogenesis of bovine pneumonic pasteurellosis: review and hypothesis. *Br. Vet. J.* 149:183-193. 10. Liebler, EM; Pohlenz, JF; Woode, GN; *et al.* (1988). Gut-associated lymphoid tissue in the large intestine of calves. I. Distribution and histology. *Vet. Pathol.* 25:503-508. 11. Magwood, SE; Barnum, DA; and Thompson, RG. (1969). Nasal bacterial flora of calves in healthy and pneumonia-prone herds. *Can. J. Comp. Med.* 33:237-243. 12. McVey, DS; Loan, RW; Purdy, CW; *et al.* 1989). Antibodies to *Pasteurella haemolytica* somatic antigens in two modes of the bovine respiratory disease complex. *Am. J. Vet. Res.* 50:443-447. 13. Mosier, DA; Simons, KR; Chengappa, MM; *et al.* (1994). Antigenic composition of *Pasteurella haemolytica* serotype-1 supernatants from supplemented on nonsupplemented media. *Am. J. Vet. Res.* 55:348-352. 14. Murray, PJ; and Young, RA (1992). Stress and immunological recognition in host-pathogen interactions. *J. Bacteriol.* 174:4193-4196. 15. Ribble, CS; Meek, AH; Janzen, ED; et al (1995). Effect of time of year, weather, and the pattern of auction market sales on fatal fibrinous pneumonia (shipping fever) in calves in a large feedlot in Alberta (1985-1988). *Can. J. Vet. Res.* 59:167-172. 16. Salyers, AA; and Whitt, DD (1994). Virulence factors that damage the host. In: *Bacterial Pathogenesis: A Molecular Approach*. pp. 47-62. ASM Press, Washington, DC. 17. Srinand, S; Ames, TR; Maheswaran, SK; *et al.* (1995). Efficacy of various vaccines against pneumonic pasteurellosis in cattle: a metaanalysis. *Prevent. Vet. Med.* 25:7-17. 18. Wilding, IR; Davis, SS; and O'Hagan, DT. (1994). Targeting of drugs and vaccines to the gut. *Pharmac. Ther.* 62:97-124.