Bovine Pneumonic Pasteurellosis: A Biotechnological Approach to Control

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Vaccination

> Never in the history of human progress has a better and cheaper method of preventing illness been developed than immunization at its best (8).

The acknowledged efficacy of vaccination in preventing major infectious diseases is based upon obvious successes such as that against smallpox, which was declared eradicated in 1980 (24), or the Clostridium sp bacterin-toxoids which are used in man and animals.

Expectations for vaccine usefulness are consequently high, but many diseases which are apparently amenable to vaccine-based control remain as significant sources of economic loss in the livestock industry. Failure to observe certain basic principles of vaccine design may have contributed to this. Vaccination is feasible and warranted if directed towards an economically important infectious disease of livestock which is caused by an identified infectious agent with known virulence-mediating determinants which induce protective immune response against a realistic challenge under usual husbandry conditions. The vaccination program must be cost-effective.

Vaccines have, in some instances, proven useful when developed empirically without specific knowledge of virulence mechanisms. Pasteur, for example, had no insight into the pathogenesis of rabies when he introduced his vaccine, and the precise immunopathogenesis of rabies remains unknown while modern rabies vaccines, oral or parenteral, attenuated or inactivated, and even recombinant types are highly efficacious. Nevertheless, the empirical approach has been distinctly unsuccessful in other instances, such as the longstanding efforts to control by vaccination respiratory disease in feedlot cattle. Efforts have been thwarted because of imprecise information on the etiological role of the many bacterial, mycoplasma, and viral agents associated with shipping fever. A further impediment has been the production of Pasteurella sp bacterins in the absence of information on the virulence-related bacterial, structural, or secreted products that should be incorporated in order to induce protective immune response. By investigation of the means by which *Pasteurella haemolytica* A1 may cause pneumonia, it has been possible to design a modern vaccine which is efficacious in field and laboratory use against the principal manifestation of shipping fever, pneumonic pasteurellosis (29,30).

If biotechnology is the provision of goods and services from cells and cell products, then the pasteurella bacterins marketed for some 50 years with the intention of preventing shipping fever were biotechnological products. In reality, however, they lacked the sophistication implied by the term "biotechnology," a word suggesting processes and products with a molecular and genetic basis. They were empirically produced in the absence of knowledge of virulence attributes of *P. haemolytica* or of how best to grow the bacterium for production of efficacious vaccines.

Pasteurella haemolytica A1 and Pathogenesis of Pneumonic Pasteurellosis

The etiological role of *Pasteurella haemolytica* A1 in shipping fever is suggested by its preponderance among bacterial, viral, or other agents isolated from the lungs of cattle dying after arrival in feedlots. In a series of 354 lungs from a group of 407,000 Colorado feedlot calves, 62% yielded pasteurella, 50% mycoplasma, 35% pasteurella and mycoplasma, and 16% bovine herpesvirus 1 (15). Typically, *P. haemolytica* serotype 1 is the most frequently isolated organism, suggesting that serotype 1 expresses virulence attributes not present in the other 14 serotypes of *P. haemolytica* (28). *Pasteurella multocida* is cultured relatively infrequently from cases of shipping fever (20) and *Haemophilus somnus*, which is sporadically isolated, has an as yet undefined role (25).

Proof of causation of pneumonic pasteurellosis by *P. haemolytica* independently of viral or other microbial agents has been obtained by intrabronchial infusion (10) or transthoracic intrapulmonary injection (23) of rapidly growing *P. haemolytica* serotype 1. Stationary growth phase bacteria, rather than those from the logarithmic phase, are relatively avirulent (2). Preinfection with the PI3 or IBR viruses, or with *Mycoplasma bovis* (12) enhances severity of pneumonia induced by *P. haemolytica*, but the bacterial dose is ultimately the determinant of severity

(35). There is no evidence that *P. haemolytica* is consistently dependent upon concomitant, or previous infection with another agent to enable it to induce severe fibrinopurulent, necrotizing bronchopneumonia.

During assembly, shipping and processing cattle for entry into feedlots, the normal nasal flora which is usually inhibitory of *P. haemolytica* serotype 1 (6) becomes permissive, and *P. haemolytica* serotype 1 rapidly predominates (9) to occur in large numbers in the tracheal air and in pneumonic lungs (13). These events are associated with the stress of transportation in an undefined fashion which may relate to the altered host physiological status reflected in high plasma cortisol levels (31) and possibly altered bacterial-nasal mucosal interactions due to host and/or bacterial cell surface changes, diminished innate or immunologically-specific clearance mechanisms, or an enhanced nutritional environment for *P. haemolytica* in the nose of shipped cattle. Experimental dual infections likely enhance the virulence of *P. haemolytica* by impairing function of the alveolar macrophage (14), the principal mediator of lung clearance and itself the target of a ruminant leucocyte-specific toxin secreted by pneumonia-inducing *P. haemolytica* (27).

The *Pasteurella haemolytica* Leucotoxin

The leucotoxin of *P. haemolytica* is a pulmonary virulence factor that is produced *in vitro* only transiently in the early logarithmic phase of bacterial growth in enriched medium (27). It impairs monocyte-generated chemotoxis (19), oxygen-dependent leucocyte mechanisms normally mediating bacterial killing (5), and is potently cytotoxic for leucocytes. Neutrophils, which rapidly enter the lung in response to *P. naemolytica* (32), induce pneumonia when killed by the bacterial leucotoxin, an effect that is abolished in neutrophil-depleted calves (31). The leucotoxin is immunogenic; toxin-neutralizing antibody detected in beef cattle is related to protection against pneumonic pasteurellosis (26) and vaccination with toxin-rich, bacterial cell-free vaccine induces protection (28,29). Definition of the leucotoxic molecule secreted by actively growing *P. haemolytica* is provided by the successful cloning and sequencing of the encoding bacterial gene (16,17).

Serotype-Specific Capsular Antigens and Other Virulence Attributes

P. haemolytica produces a surface capsule in phases of active growth coincident with its enhanced production of leucotoxin and of fimbria (7,21). The capsule and fimbriae are recognized in electron micrographs of the bacteria within lungs of cattle with pneumonic pasteurellosis (22), suggesting that these attributes of the bacterium growing rapidly *in vitro* are also expressed *in vivo* as the bacterium proliferates.

The *P. haemolytica* capsule carrying antigenic determinants which define the serotype specificity of the bacterium and immune response to these antigens is, together with response to the leucotoxin, a component of protective immunity (29). Genes encoding the serotype-specific antigen of *P. haemolytica* A1 have also been cloned (11) and the resulting recombinant *E. coli* express the *P. haemolytica* A1 antigen.

The possible role of the fimbriae of *P. haemolytica* (21,22) as virulence factors has not been elucidated but it is likely that both fimbriae and capsule exert surface adhesion and antiphagocytic effects. *P. haemolytica* is not readily phagocyitized by bovine lung macrophages in the absence of surface antigen-specific antibody (18). Resistance to complement-mediated lysis by *P. haemolytica* isolated from pneumonic lungs and the relative susceptibility of nasal isolates to killing by the classical complement activation pathway (4) may indicate an additional virulence mechanism. It is not known if this functional attribute of *P. haemolytica* is due to capsule, fimbriae or to as yet unidentified structural components.

A Bacterial Cell Free *Pasteurella haemolytica* A1 Vaccine

With knowledge of at least two of the virulence factors, leucotoxin and serotype-specific antigens of *P. haemolytica*, their molecular nature and the conditions of *in vitro* growth which optimize their production and retrieval, it has been possible to develop and manufacture a vaccine which incorporates these molecules while excluding bacterial cells (29,30). The latter is important since untoward reactions to the traditional whole cell pasteurella bacterins may have been due to their high content of endotoxin; an expected result of growing cultures of Gram negative bacteria to maximum cell yield in bacterin production rather than defining growth to optimize yield of specific virulence-related molecules (27). The balanced inclusion in the vaccine of serotype-specific antigens and the leucotoxin avoids the adverse reaction observed in some *P. haemolytica*-challenged cattle which lack effective leucotoxin-neutralizing antibody but have bacterial surface antigen-specific antibody (33,3). This reaction is likely the result of surface-antigen-specific antibody promoting phagocytosis of actively leucotoxin-secreting *P. haemolytica* in the absence of toxin-neutralizing antibody such that leucocyte killing, and subsequently development of pneumonia, are facilitated (34).

Using laboratory challenge with *P. haemolytica* A1 to test vaccine efficacy, the leucotoxin and serotype-specific antigen-rich bacterial extract, Presponse™* induces highly significant protection as assessed by clinical and necropsy examination (30). A trial involving 22 vaccinates (days 0

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and 21) and 10 unvaccinated control calves using intratracheal challenge (day 42) with 25 ml containing 1.5 x 10^9 bacteria/ml indicated significantly ($p<0.01$) reduced composite clinical scores (dyspnea, cough, nasal discharge, inappetence, lethargy, recumbancy) and severity of pneumonia at necropsy ($p<0.001$) based upon a composite lung lesion scoring system. Results are summarized in Tables 1 and 2. While 32% of vaccinated calves had recognizable lesions (score >3), 80% of control calves developed pneumonia with a preponderance of individuals having severe lesion grades (Table 2). Vaccine efficacy (30) was calculated by Abbot's correlation, P=(Pv-Pc) x 100 where

P = percent efficacy
Pv = proportion of vaccinates without moderate to severe pneumonia
Pc = proportion of controls without moderate to severe pneumonia

TABLE 1. Response to P. Haemolytica Challenge of Vaccinated (22) and Control (10) Calves (Trial XV).

	Vaccinates	Controls	p
Clinical[1] Scores	1.4+/-1.3	3.2+/1.5	<0.01
Respiratory[2] Rates	2.6+/-2.0	4.6+/-1.26	<0.01
Temperature[2]	2.2+/-2.2	3.2+/-2.0	N.S.
Lung Lesion[3] Score	2.8+/-2.9	6.9+/-2.9	<0.001
Isolation of *P. haemolytica*	13/21 (62%)	10/10 (100%)	
Correlation of Clinical and Lesion Scores	r = 0.91		

N.S. = no significant difference (Chi square)
[1]mean daily score/calf for 5 days of observation, maximum 5
[2]number of days with increased respiratory rate, maximum 5
[3]mean group score; maximum score of 10/calf

TABLE 2. Pneumonia in P. Haemolytica Challenged and Control Calves (Trial XV)

	Lesion Grade[1] and Frequency											Mean
	0	1	2	3	4	5	6	7	8	9	10	
C	0	0	1	1	1	1	0	1	1	1	3	6.9
V	7	2	5	1	0	1	4	0	1	1	0	2.8

C = unvaccinated controls
V = 2x vaccinated prior to challenge
[1]severity and extent of pneumonia at necropsy. Lesions grade 3 and under are considered to be essentially normal lungs.

On the basis of lung lesions, this indicates 60.2% efficacy. *P. haemolytica* was isolated at necropsy from 10/10 and 13/21 lungs of unvaccinated and vaccinated calves.

Field trials of Presponse™ administered to calves arriving in the feedlot have been conducted by Drs. K. Jim and T. Guichon (Okotoks, Alta) and the results of these trials are summarized in Table 3. Significant protection was afforded by vaccination with a calculated return/100 animals vaccinated of Cdn. $1,632.00.

TABLE 3. Presponse Field Trial, Alberta, 1987–1988

	Vaccinates (781)	Controls (1291)	p
Mortality	2.2% (17)	4.3 (56)	<0.01
Fibrinous Pneumonia	1.15% (9)	2.32% (30)	<0.05
Other	1.02% (8%)	2.01% (26)	<0.05
Initial Treatment	56.9%	59.3%	N.S.
Relapse 1	19.8% (155)	23.47% (303)	<0.05
Relapse 2	7.17% (56)	9.2% (119)	<0.05
Total Days Treatment	2064	3715	<0.05
Treatment Days/Calf	2.64	2.88	<0.05

N.S. = no significant difference

Vaccinated and control groups of 781 and 1291 individuals were obtained by randomly assigning calves upon their arrival in a 20,000 head capacity feedlot. Calves ranging from 560 to 827 lb. were received between October 1 and December 14 in groups of 4 to 111, processed and added to pens to a maximum of 300 animals. Processing included administration of clostridial, *Haemophilus somnus* and IBR/PI3 vaccines, anabolic steroids, Vitamins A, D and E, ivermectin and oxytetracycline. Each calf was branded and identified by ear tag. The *P. haemolytica* vaccine was given by intramuscular injection during processing. A second injection of Presponse™ was given when temperatures were checked between 1 and 5 days after arrival. Cattle with signs of respiratory disease and with a rectal temperature above 104°F were treated with antibiotics.

The initial treatment rates for animals in the vaccinated and control groups were 56.9% and 59.3% respectively. Vaccine efficacy was assessed by initial treatment rate, number of relapses, number of treatment days, mortality and incidence of fibrinous pneumonia or other disease. In all but the initial treatment rate, vaccinated and control cattle differed significantly ($p<0.01$ - $p<0.05$, chi square, Table 3).

Conclusions

A critical evaluation of the microbial agents apparently

causing shipping fever and of the virulence attributes of the most incriminated bacterium, *P. haemolytica* A1, together with development of manufacturing processes which select and preserve the immunogenicity of the virulence-related antigens, has resulted in an apparently efficacious vaccine against pneumonic pasteurellosis.

Insofar as Presponse™ is based upon knowledge of at least two of the molecular mediators of virulence produced by *P. haemolytica*, and its manufacture involves a relatively sophisticated system of molecular enrichment, it may fairly be considered a product of "biotechnology" with the connotation of science and design having displaced empiricism in its evolution to the marketplace.

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