Feedlot Session I

"Bacterial Pathogens in the Feedyard - - Pathology and Prevention"

Moderator— J.P. Pollreisz

Evaluation of *Pasteurella* and *Haemophilus* Vaccines

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Introduction

The family Pasteurellaceae Pohl contains gram-negative, facultatively anaerobic and fermentative bacteria of the genera *Pasteurella, Haemophilus*, and *Actinobacillus*. Of these genera, *Haemophilus somnus, Pasteurella multocida* and *Pasteurella haemolytica* are prominent pathogens in domestic cattle causing severe diseases and major economic losses. Mechanisms of immunity to these bacteria have been difficult to determine, and efficacious vaccines have been a challenge to develop and evaluate.

In this review, we will summarize current published data on antigens and immunity associated with *H. somnus, P. multocida, and P. haemolytica.* This will be followed by a discussion of experimental studies conducted in our laboratories using commercial *P. haemolytica* vaccines.

Haemophilus somnus Antigens and Immunity

H. somnus causes thromboembolic meningoencephalitis (TEME), septicemia, fibrinous pneumonia, arthritis, infertility, vulvovaginitis, and abortion in cattle. Reservoirs for *H. somnus* appear to be the respiratory and urogenital tracts of cattle. *H. somnus* can cause severe fibrinous pneumonia in feedlot cattle; however, prevalence is substantially less than that for *Pasteurella sp.*[1] *H. somnus* isolates may have minor differences in antigenic profiles. Those differences are irrespective of anatomic location (such as nervous, urogenital or respiratory systems) from which the isolates were obtained.

H. somnus is readily ingested by bovine neutrophils and macrophages; however, it is poorly killed by those cells.[2] The organism also has the ability to replicate in bovine macrophages and to inhibit myeloperoxidase-dependent killing by bovine neutrophils. These bacterial properties make *H. somnus* a formidable opponent to host defenses.

Potential immunogens have been studied in *H. somnus* and consist of lipopolysaccharide (LPS), outer membrane proteins (OMPs), and iron-regulated proteins. As with most Gram-negative bacteria, the polysaccharide moiety (O-antigen) of *H. somnus* LPS is a dominant antigen which stimulates an antibody response following natural or experimental exposure.[3] Currently, there is no evidence that those anti-LPS antibodies are protective. In fact, Primal *et al.*[4] demonstrated that cattle vaccinated with an LPS-free protein fraction of *H. somnus* were protected against experimental intrapulmonic challenge. However, cattle vaccinated with the protein fraction supplemented with homologous LPS were not protected against challenge and had greater lesions than in the controls. Antibody responses to the lipid A moiety (endotoxin) of LPS have been poorly characterized for most gram-negative bacteria. Inzana and Todd[5] demonstrated that cattle and mice vaccinated with a *H. somnus* lipid A-protein conjugate developed anti-lipid A antibodies. Vaccinated mice, however, were not protected against *H. somnus* challenge, and cattle were not challenged.

The literature contains descriptions of the characterization and cloning of numerous OMPs from *H. somnus*. The major OMP of *H. somnus* is the 40 kDa surface exposed protein.[3] Convalescent sera from cattle after recovery from experimental *H. somnus* pneumonia demonstrated intense reactions against 78 and 40 kDa OMPs.[3] Monospecific antibodies to the 40 kDa OMP were associated with resistance of cattle to pneumonia. Cole *et al.*[6] demonstrated that a 76 kDa OMP encoded by *H. somnus* was associated with the bacterium's resistance to serum killing. Interestingly, an antigenic extract of *H. somnus* that was shown to protect cattle against TEME did not contain either the 40 or 78 kDa OMPs; therefore, the role of specific OMP in stimulating immunity is still undetermined. Several other immunogenic OMPs of *H. somnus* have been described, including two proteins that bind immunoglobulins and interfere with complement-mediated lysis and a heat-modifiable OMP structurally related to the major structural OMP of *E. coli* (OmpA).[7] The relationship between those OMPs and immunity has not been established.

Three iron-regulated proteins (105, 85, and 73 kDa) have been described in *H. somnus* grown under conditions of iron restriction.[8] Iron-regulated proteins of *H. somnus* bind to bovine transferrin and are used by the bacterium to acquire iron within the iron-restricted host environment. Expression of iron-regulated proteins seems to vary among *H. somnus* isolates, and the iron-regulated proteins expressed in several isolates from pneumonia were not recognized by antibodies present in hyperimmune serum generated against a bacterium isolated from a case of septicemia. Therefore, strain variations in *H. somnus* may have important implications in development of efficacious vaccines.

The *H. somnus* biologicals available consist of bacterins and extract vaccines. Efficacy for these vaccines has been generally favorable but variable. Overall, vaccine-induced immunity has been best against experimental and natural TEME.[9] Using an experimental challenge, Groom and Little[10] demonstrated significant protection afforded calves vaccinated twice with a *H. somnus* bacterin. Resistance correlated with a high antibody response. Under field conditions, commercial *H. somnus* bacterins have produced variable effects with respect to protection against pneumonia.[11-12] For instance, in an Ontario study the authors noted that using a *H. somnus* bacterin was strongly associated with no or low mortality in feedlots. In two other field trials, no reduction in mortality or numbers of animals treated could be demonstrated. Ribble *et al.*[11] demonstrated that *H. somnus* vaccination reduced steer mortality but not heifer mortality in feedlots. In that and another trial,[13] one vaccination was as effective as two in reducing mortality.

Pasteurella multocida Antigens and Immunity

P. multocida is classified into 5 capsular serogroups (A, B, D, E, and F) and 16 somatic serotypes (1-16).[14] *P. multocida* serogroups B and E are associated with bovine hemorrhagic septicemia, whereas bovine respiratory diseases are mainly associated with serogroup A.[15] Hemorrhagic septicemia is not an important disease in the United States; however, more is known about immunity to hemorrhagic septicemia than to *P. multocida*-induced pneumonia. *P. multocida* serogroup A is most commonly associated with a fibrinous bronchopneumonia of feedlot cattle that is less prevalent and fulminating than the fibrinous pleuropneumonia associated with *P. haemolytica* infection.[1] *P. multocida* can also be isolated from enzootic pneumonia of dairy and beef calves less than 6 months old.[15]

Potential *P. multocida* immunogens are capsular polysaccharide, LPS, OMPs, and iron-regulated proteins. *P. multocida* capsular polysaccharide is a hapten and does not appear to stimulate protective immunity. Purified *P. multocida* LPS is antigenic; however, the extent of antibody response following immunization depends on animal species inoculated, LPS type used, and route and method of inoculation.[14] In addition, protection afforded by immunization with *P. multocida* LPS is somewhat animal species dependent. In general, *P. multocida* LPS seems to be a major immunogen in birds; however, mice, cattle, and rabbits have not been consistently protected against *P. multocida* infection following immunization with LPS.[16] Vaccination with LPS-protein complexes have induced some protection in several animal species. Recently, protection of mice vaccinated with *P. multocida* LPS-protein complexes was found to be due to OMPs in the complexes.[17]

Recent studies showed that mice vaccinated with *P. multocida* outer membranes were reasonably protected against challenge; however, outer membranes expressing iron-regulated proteins were more effective immunogens.[18] The immunogenic role of specific *P. multocida* OMPs has been best characterized in rabbits. Lu *et al.*[19] demonstrated that rabbits mounted major antibody responses against 5 *P. multocida* OMPs (27, 37.5, 49.5, 58.7, and 64.4 kDa). They further demonstrated that vaccination with *P. multocida* outer membranes protected rabbits against homologous challenge, and protection was due to antibodies against OMPs and not LPS. More specifically, a monoclonal antibody against a 37.5 kDa OMP protected both mice and rabbits against *P. multocida* challenge.

Biologicals available for *P. multocida* in cattle are bacterins and live vaccines, usually in combination with *P. haemolytica*. The efficacy of these biologicals in protecting cattle against pneumonia has not been clearly established. Experimental extract vaccines have been

found to protect against hemorrhagic septicemia but not against pneumonia.[20] Because of the difficulty in protecting mice and cattle with *P. multocida* LPS, the similarity between *P. multocida*-induced respiratory disease in cattle and that seen in the rabbit, and the potential of OMP and outer membranes expressing iron-regulated proteins to protect against experimental pasteurellosis, OMP and iron-regulated OMP should be investigated as potential immunogens for cattle.

Pasteurella haemolytica Antigens and Immunity

P. haemolytica can be classified according to capsular antigens into 16 serotypes.[15] Furthermore, *P. haemolytica* isolates can be biotyped as A or T based on colony morphology and carbohydrate fermentation. Recent studies of OMP and the genome of *P. haemolytica* demonstrated marked differences in electrophoretic patterns and nucleic acid sequences between the A and T biotypes.[21] *P. haemolytica* biotype T has been reclassified as *Pasteurella trehalosi* and will not be considered in this review. Various serotypes of *P. haemolytica* biotype A, especially serotype 1, are responsible for the severe fibrinous pleuropneumonia of feedlot and stocker cattle known as shipping fever.

P. haemolytica has numerous potential immunogens. Those with the most potential for stimulating immunity include capsular polysaccharide, LPS, OMPs, fimbriae, iron-regulated proteins, and a secreted leukotoxin (LKT).[22] Two approaches have been used for determining the importance of various immunogens for stimulation of immunity to *P. haemolytica*. First is the vaccination of cattle, sheep, or goats with purified or relatively purified antigens followed by challenge with virulent *P. haemolytica*. The second approach uses sera from cattle previously vaccinated with various biologicals and challenged. The antibody responses to specific antigens are quantified and statistically correlated with the lesion score obtained after challenge. Thus, a significant correlation between a high antibody response and resistance to challenge can potentially be used as a predictor of the importance of an antigen in stimulating immunity.

Antibody responses to *P. haemolytica* fimbriae have not been documented. *P. haemolytica* A1 capsular polysaccharide is a virulence factor that interferes with phagocytosis and killing of *P. haemolytica* and complement-medicated bacteriolysis.[2,23] Immunization of ruminants with *P. haemolytica* capsular polysaccharide or live or killed whole cell preparations results in an antibody response to the capsule. Antibody responses to *P. haemolytica* capsular polysaccharide inconsistently correlated with resistance to experimental challenge in calves vaccinated with various experimental vaccines.[24] Recently, Conlon and Shewen[25] reported that vaccination of calves with purified capsular polysaccharide was ineffective in protecting calves against *P. haemolytica* challenge even when recombinant LKT was included in the experimental vaccine.

P. haemolytica LPS has classical endotoxin function *in vivo* and it can alter leukocyte function and is toxic to bovine endothelial cells *in vitro*.[26] Antibody responses to the LPS O-antigen are readily detected in calves vaccinated with live and killed *P. haemolytica* biologicals; however, the intensity of antibody responses to LPS did not correlate with resistance to experimental challenge.[27] Antibody responses to the toxic lipid A moiety have not been demonstrated.

The antibody responses to *P. haemolytica* OMPs have been incompletely characterized. Resistance to experimental challenge was enhanced by vaccination with surface extracts of *P. haemolytica*, and antibody responses to protein antigens in those extracts correlated with resistance.[24] Mosier *et al.*[28] showed that high antibody responses to several proteins in the surface extract correlated with resistance to experimental challenge. The highest correlations were for antibody responses to proteins with molecular masses of 86, 66, 49, and 31 kDa. Several of those proteins have molecular masses equivalent to major OMPs of *P. haemolytica*. Recently, we found that vaccination of cattle with *P. haemolytica* OMP-enriched preparations induced serotypic immunity against experimental challenge.

Iron-regulated proteins of *P. haemolytica* have been described. The 100, 77, and 70 kDa proteins are located in the outer membrane of *P. haemolytica* grown *in vivo* or under iron-restricted *in vitro* conditions.[29] A 35 kDa iron-regulated periplasmic protein has been demonstrated in *P. haemolytica* A2.[30] Gilmour *et al.*[31] demonstrated that sodium salicylate extract vaccines of *P. haemolytica* A2 prepared from bacteria grown under iron-restricted conditions protected sheep better than similar vaccines prepared from *P. haemolytica* grown in media containing iron and not expressing the iron-relegated proteins. Studies in one of our laboratories demonstrated that cattle vaccinated with live *P. haemolytica* A1 developed significant antibody responses to the 70 and 77 kDa iron-regulated proteins; however, there was no significant correlation between antibody responses to those proteins and resistance to experimental challenge.[32]

The *P. haemolytica* LKT has received much acclaim as an immunogen against pneumonic pasteurellosis of cattle. The LKT is a member of the RTX family of toxins, is lytic for ruminant leukocytes at high concentrations, and activates neutrophils at low concentrations. LKT is, therefore, thought to have a major role in the pathogenesis of *P. haemolytica*-induced pneumonia.[33] The genes for LKT have been cloned and sequenced. Antibodies to LKT are not *P. haemolytica* serotype spe-

cific and could potentially offer some protection against heterologous serotypes. Vaccination of cattle with live *P. haemolytica* or supernatants from logarithmic-phase *P. haemolytica* cultures induced neutralizing antibody titers to LKT, and high antibody titers correlated with resistance to experimental or natural infection with *P. haemolytica*.[22] Shewen and Wilkie[34] demonstrated that antibodies to surface antigens as well as to LKT were important in inducing protection in experimental pneumonic pasteurellosis. Conlon and Shewen[35] demonstrated that the addition of recombinant LKT to a supernatant vaccine enhanced protection against experimental challenge. In our laboratories and others, however, protection against experimental challenge was seen in cattle vaccinated with *P. haemolytica* biologicals that did not contain LKT and neutralizing antibodies to LKT were not detected.[22]

Currently available *P. haemolytica* biologicals are modified-live vaccines, bacterins, bacterial surface extracts, and culture supernatants. Some of the latter biologicals contain LKT and others do not. Most biologicals in the U.S. contain *P. haemolytica* A1; however, *P. haemolytica* T3 and T4 are occasionally included in bacterins. Live *P. haemolytica* vaccines protect cattle well against experimental pneumonic pasteurellosis, but they can have undesirable side effects such as fever, localized abscesses, and lameness.[36] Zeman *et al.*[37] described 11 cases of *P. haemolytica* septicemia in calves vaccinated with a commercial live *P. haemolytica* vaccine. Bacterins with oil adjuvants induced protective immunity against experimental bovine pneumonic pasteurellosis;[27] however, most commercial *P. haemolytica* bacterins do not contain LKT and have aluminum hydroxide as an adjuvant. Those bacterins are usually poor immunogens and do not afford good protection. Because of possible side effects or lack of efficacy of *P. haemolytica* live vaccines and certain bacterins, many of the newer *P. haemolytica* biologicals are using the subunit, extract, or culture supernatant approach and many contain LKT. Those biologicals are efficacious against experimental challenge. Results have been variable, however, when newer *P. haemolytica* biologicals have been tested under field conditions.

Experimental Evaluation of *P. haemolytica* Biologicals

We have evaluated two commercial *P. haemolytica* vaccines (Septimune[R]PH-K[Fort Dodge Laboratories' bacterin-solubilized surface antigens] and One Shot™ [SmithKline Beecham Animal Health's bacterin toxoid]), using a transthoracic challenge model.[38] Although this is an unnatural route of challenge, it consistently produces quantifiable pneumonia in 400-600 lb. beef cattle without introducing variables such as viruses or a stressor. Results of many of our previous experimental vaccine studies have been corroborated by others using combination respiratory virus-*P. haemolytica* challenges in neonatal or weanling calves.

In two experiments, 300-450 lb. beef cattle were vaccinated with one of two new generation *Pasteurella haemolytica* vaccines, Septimune [R]PH-K or One Shot™, and challenged intrathoracically with virulent *P. haemolytica*. Injection of either vaccine caused no adverse local or systemic reactions.

In Experiment 1, cattle were vaccinated intramuscularly with either full or 1/10 doses of Septimune[R]PH-K. Two vaccinations 21 days apart stimulated significant increases ($p < 0.05$) in serum antibodies to *P. haemolytica* surface antigens. Two positive control calves were vaccinated with live *P. haemolytica*. Only those 2 calves developed LKT neutralizing antibodies. After challenge on day 35, 25% of saline-vaccinated control cattle died and surviving cattle developed moderate to severe pneumonia (mean lesion score of 12.6 ± 6.0 out of a maximum score of 20). Vaccinated cattle had transient clinical signs of respiratory disease and no deaths. Pulmonary lesions were 64.5 - 71.4% less ($p < 0.05$) than for saline-vaccinated controls (mean lesion scores 3.6 - 4.5).

In Experiment 2, a single dose of One Shot™ given by either intramuscular or subcutaneous route stimulated significant increases ($p < 0.05$) in serum antibodies to *P. haemolytica* surface antigens and leukotoxin. Challenge was with a *P. haemolytica* dose nearly 2 times greater than in Experiment 1. Eighty percent of saline-vaccinated control cattle and 10% of vaccinated cattle died. All saline-vaccinated controls had severe pneumonia (mean lesion score of 18.1 ± 4.1). The surviving One Shot ™-vaccinated cattle had transient clinical signs of respiratory disease. Pulmonary lesions for One Shot™-vaccinates were 52.5 - 53.0% less than for saline controls (mean lesion scores or 8.5 - 8.6). Vaccination with either Septimune[R]PH-K or One Shot™ significantly enhanced resistance against experimental *P. haemolytica* challenge.

In our previous experiments using experimental or commercial *P. haemolytica* biologicals, we found significant correlations between serum antibodies to surface antigens and resistance to challenge. We, therefore, undertook nonchallenge studies to determine the duration of antibody responses induced by commercial *P. haemolytica* biologicals and the effects of revaccination after extended periods.

In our first nonchallenge study, 9 calves were vaccinated with One Shot™ on day 0. Antibody responses to surface antigens were followed for 42 days. Antibodies to surface antigens were detectable on day 7 after vaccination. Those responses were significantly greater ($p < 0.05$) than for nonvaccinated controls on days 10-21. Between days 28 and 42, however, antibody re-

sponses were only insignificantly greater ($p > 0.05$) than for controls.

In the second nonchallenge study, calves were vaccinated with either Discovery^R-4+PH (Franklin Laboratories' vaccine containing killed viruses and *P. haemolytica* bacterin-solubilized surface antigens) on days 0, 28, and 140, One Shot ™ on days 0 and 140, or Presponse^R [American Cyanamid's culture supernatant vaccine containing leukotoxoid and surface antigens] on days 0, 28, 140. Antibody responses were determined through day 196. For the One Shot™-vaccinated group, antibody responses to *P. haemolytica* surface antigens were significantly greater ($p < 0.05$) on days 7 and 21 than for the controls, whereas antibody responses induced by Discovery^R-4+PH or Presponse^R were not significantly greater ($p > 0.05$) than controls at that time. Revaccination of calves with Discovery^R-4+PH or Presponse^R on day 28 resulted in antibody responses that were significantly greater ($p < 0.05$) than controls on day 42. Revaccination at day 140 resulted in significant anamnestic responses ($p < 0.05$) on day 154 for each vaccine group. Antibody responses remained significantly greater ($p < 0.05$) than for controls on days 168 and 196 for the Discover^R-4+PH and One Shot™ groups but not for the Presponse^R vaccinated group.

Conclusions

Excellent progress has been made in *P. haemolytica* vaccine technology in recent years. Understanding of important immunogens has been greatly enhanced through experimental studies in many countries. Several commercially available *P. haemolytica* biologicals seem to have the potential to reduce losses to pneumonic pasteurellosis. Understanding of important immunogens for protection of cattle against *P. multocida* infection has not, however, kept pace with that for *P. haemolytica* nor has the efficacy of current bacterins been seriously examined. Although understanding of potential immunogens of *H. somnus* has greatly increased in recent years, that technology has not been incorporated into newer biologicals. The efficacies of current *H. somnus* biologicals against respiratory diseases have not been firmly established.

References

1. Welsh, R.D., 1993. Bacterial and *Mycoplasma* species isolated from pneumonic bovine lungs. *Agri-Pract.*, 14: 12-16. 2. Czuprynski, C.J. and Sample, A.K., 1990. Interactions of *Haemophilus-Actinobacillus-Pasteurella* bacteria with phagocytic cells. *Can. J. Vet. Res.*, 54: S36-S40. 3. Gogolewski, R.P., Kania, S.A., Inzana, T.J., Widders, P.R., Liggett, H.D. and Corbeil, L.B., 1987. Protective ability and specificity of convalescent serum from calves with *Haemophilus somnus* pneumonia. *Infect. Immun.*, 55: 1403-1411. 4. Primal, S.V., Silva, S.V. and Little, P.B., 1990. The protective effect of vaccination against experimental pneumonia in cattle with *haemophilus somnus* outer membrane antigens and interference by lipopolysaccharide. *Can. J. Vet Res.* 54: 326-330. 5. Inzana, T.J. and Todd, J., 1992. Immune response of cattle to *haemophilus somnus* lipid A-protein conjugate vaccine and efficacy in a mouse abortion model. *Am. J. Vet. Res.*, 53:175-179. 6. Cole, S.P., Guiney, D.G. and Corbeil, L.B., 1993. Molecular analysis of a gene encoding a serum-resistance-associated 76 kDa surface antigen of *Haemophilus somnus*. *J. Gen. Microbiol.*, 139: 2135-2143. 7. Won, J. and Griffith, R.W., 1993. Cloning and sequencing of the gene encoding a 31-kilodalton antigen of *Haemophilus somnus*. *Infect & Immun.*, 61: 2813-2821. 8. Wedderkopp, A., Primal, S.V., Silva S. and Little, P.B., 1993. Differences in protein expression of *Haemophilus somnus* grown under conditions of iron-restrictions. *Vet. Microbiol.*, 35: 91-100. 9. Stephens, L.R., Little, P.B., Wilkie, B.N. and Barnum. D.A., 1984. Isolation of *Haemophilus somnus* antigens and their use as vaccines for prevention of bovine thromboembolic meningoencephalitis. *Am. J. Vet. Res.*, 45: 234-239. 10. Groom, S.C. and Little, P.B., 1988. Effects of vaccination of calves against induced *Haemophilus somnus* pneumonia. *Am. J. Vet. Res.*, 49: 793-800. 11. Ribble, C.S., Jim, G.K. and Janzen, E.D., 1988. Efficacy of immunization of feedlot calves with a commercial *Haemophilus somnus* bacterin. *Can. J. Vet. Res.*, 52: 191-198. 12. Martin, W., Ocres, S., Janzen, E., Willson, P. and Allen, B., 1984. A field trial of preshipment vaccination of calves. *Can. Vet. J.*, 25:145-147. 13. Van Donkersgoed, J.V., Schumann, F.J., Harland, R.J., Potter, A.A. and Janzen, E.D., 1993. The effect of route and dosage of immunization on the serological response to a *Pasteurella haemolytica* and *Haemophilus somnus* vaccine in feedlot calves. *Can. Vet. J.*, 34: 731-735. 14. Rimler, R.B. and Rhoades, K.R., 1989. *Pasteurella multocida*. *In:* C. Adlam and J. M. Ritter (Editors) *Pasteurella* and Pasteurellosis. Academic Press, London, pp. 37-73. 15. Frank, G. H., 1989. Pasteurellosis of cattle. *In:* C. Adlam and J.M. Ritter (Editors), *Pasteurella* and Pasteurellosis. Academic Press, London, pp. 197-222. 16. Mosier, D., 1993. Prevention and control of pasteurellosis. In Patten, B.E., Spencer, T.L., Johnson, R.B., Hoffman, D. and Lahave, L. (eds.) *Pasteurellosis in Production Animals*. A CIAR Proceedings, Brisbane, pp. 121-134. 17. Muniandy, N. and Mukkur, T.K.S., 1993. Protective potential of purified lipopolysaccharide versus conjugated oligosaccharide of *Pasteurella multocida* type B in mice. In Patten. B.E. Spencer, T. L. Johnson, R.B., Hoffman, D. and Lahave, L. (eds.). *Pasteurellosis in Production Animals*. A CIAR Proceedings, Brisbane, pp. 149-155. 18. Kennett, L., Muniandy, N., Mukkur, T.K.S., 1993. Comparative protective potential of non-living intact cells and purified outer membranes and associated proteins of *Pasteurella multocida* type 6:B grown under iron-regulated conditions. In Patten, B.E., Spencer, T.L., Johnson, R.B., Hoffman, D. and Lahave, L. (eds.). *Pasteurellosis in Production Animals*. A CIAR Proceedings, Brisbane, pp. 144-148. 19. Lu, Y.S., Lai, W.C., Pakes, S.P. and Stefanu, C., 1991c. The outer membrane of *Pasteurella multocida* 3:A protects rabbits against homologous challenge. *Infect. Immun.*, 59: 4517-4523. 20. Erler, W. and Schonkerr, W., 1993. Immunogenic effect of *Pasteurella multocida* extracts in calves against pasteurella infections. In Patten, B.E., Spencer, T. L., Johnson, R.B., Hoffman, D. and Lahave, L. (eds.). *Pasteurellosis in Production Animals*. A CIAR Proceedings, Brisbane, pp. 156-158. 21. Dewhirst, F.E., Paster, B.J., Olsen, I. and Fraser, G.J., 1992. Phylogeny of 54 representative strains of species in the family *Pasteurellaceae* as determined by comparison of 16SrRNA sequences. *J. Bacteriol.*, 174: 2002-2013. 22. Confer, A.W., 1993. Immunogens of *Pasteurella*. *Vet. Microbiol.*, 37: 353-368. 23. Chae, C.H., Gentry, M.J., Confer, A.W. and Anderson, G.A., 1990. Resistance to host immune defense mechanisms afforded by capsular material of *Pasteurella haemolytica*, serotype 1. *Vet. Microbiol.*, 25: 241-251. 24. Confer, A.W., Simons, K.R., Panciera, R.J., Mort, A.J., and Mosier, D.A., 1989. Serum antibody response to carbohydrate antigens of *Pasteurella haemolytica* serotype 1: relation to experimental bovine pneumonic pasteurellosis. *Am. J. Vet. Res.*, 50:98-105. 25. Conlon, J.A.R., and Shewen, P.E., 1993. Clinical and serological evaluation of a *Pasteurella haemolytica* A1 capsular polysaccharide vaccine. *Vaccine*, 11: 767-772. 26. Confer, A.W., Panciera, R.J., Clinkenbeard, K.D. and Mosier, D.A., 1990. Molecular aspects of viru-

lence of *Pasteurella haemolytica*. *Can. J. Vet. Res.*, 54 Suplmt:S48-S52. 27. Confer, A.W., Panciera, R.J. and Mosier, D.A., 1986. Serum antibodies to *Pasteurella haemolytica* lipopolysaccharide: relationship to experimental bovine pneumonic pasteurellosis. *Am. J. Vet. Res.*, 47: 1134-1138. 28. Mosier, D.A., Simons, K.R., Confer, A.W., Panciera, R.J. and Clinkenbeard, K.D., 1990 *Pasteurella haemolytica* antigens associated with resistance to pneumonia pasteurellosis. *Infect. Immun.* 57: 711-716. 29. Donachie, W. and Gilmour, N.J.L., 1988. Sheep antibody response to cell wall antigens expressed *in vivo* by *Pasteurella haemolytica* serotype A2. *FEMS Microbiol. Let.*, 56: 271-276. 30. Lainson, F.A., Harkins, D.C., Wilson, C.F., Sutherland, A.D., Murray, J.E., Donachie, W. and Baird, G.D., 1991. Identification and localization of an iron-regulated 35 kDa protein of *Pasteurella haemolytica* serotype A2,. *J Gen. Microbiol.*, 137: 219-226. 31. Gilmour, N.J.L., Donachie W., Sutherland. A.D., Gilmour, J.S., Jones, G.E. and Quirie, M., 1991. Vaccine containing iron-regulated proteins of *Pasteurella haemolytica* A2 enhances protection against experimental pasteurellosis in lambs. *Vaccine*, 9: 137-139. 32. Confer, A.W., McCraw, R.D., Durham, J.A., Morton, R.J. and Panciera, R.J., 1994. Serum antibody response of cattle to iron-regulated outer membrane proteins of *Pasteurella haemolytica* A1. *Vet. Immonopathol.*, in press. 33. Lo, R.Y.C., 1990 Molecular characterization of cytotoxins produced by *Haemophilus, Actinobacillus, Pasteurella*. *Can. J. Vet. Res.* 54 Supplm.:S33-S35. 34. Shewen, P.E. and Wilkie, B.N., 1988. Vaccination of calves with leukotoxic culture supernatant from *Pasteurella haemolytica*. *Can. J. Vet. Res.*, 52: 30-36. 35. Conlon, J.A. and Shewen, P.E., 1991. Efficacy of recombinant leukotoxin in protection against pneumonic challenge with live *Pasteurella haemolytica* A1. *Infect. & Immun., 59: 587-591.* 36. Confer, A.W., Panciera, R.J., Gentry, M.J. and Fulton, R.W., 1986. Immunologic response and resistance to experimental pneumonic pasteurellosis in cattle vaccinated with varying doses of lyophilized *Pasteurella haemolytica*. *Am. J. Vet. Res.*, 47: 1853-1857. 37. Zeman, D., Neiger, R., Nietfield, J., Miskimins, D., Libal, M., Johnson, D., Janke, B., Gates, C. and Forbes, K., 1993. Systemic *Pasteurella haemolytica* infection as a rare sequel to avirulent live *Pasteurella haemolytica* vaccination in cattle. *J. Vet. Diagn. Invest.*, 5: 555-559. 38. Panciera, R.J. and Corstvet, R.E., 1984. Bovine pneumonic pasteurellosis: model for *Pasteurella haemolytica*- and *Pasteurella multocida*-induced pneumonia in cattle. *Am. J. Vet. Res.*, 45: 2532-2537.

Technology Transfer Symposium

Design of Strategic Anthelmintic Control Programs in Cattle Using a Mathematical Model: *Paraban*

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The design of cost-effective practical parasite control programs is fundamental to achieving optimum productivity from grazing cattle. Strategic treatment regimens such as the 3, 8 and 13 program utilizing ivermectin or the 3 and 6 recommendation for conventional anthelminitics have been developed from systematic field trials. These recommendations are relatively inflexible as they do not allow for altered animal management schemes. The objective for development of the PARABAN program was to facilitate design of efficient parasite control measures that would account for distinct husbandry practices and allow recognition of the potential consequences of missed or altered timing of doses. Data on the population biology of *Cooperia, Trichostrongylus, Haemonchus* and *Ostertagia* permit the development of a model for the processes that regulate and control parasite abundance. Input of local climatic data, as well as locally identified patterns of inhibition, allow tailoring of the model to project epidemiology on a regional basis. Incorporation of data on anthelmintic efficacy allows comparison of different treatment strategies, according to management objectives identified by the user. Validation studies completed in Europe and South America and comparison of model projections with published field data from the United States and New Zealand suggest that the model has a sound base for use throughout the world as a guide in development of parasite control strategies. Informed use of such a model will provide an educational basis to facilitate responsible and cost effective use of anthelminitics.