Conclusions

The SVANOVA ELISA for BLV and BVD appear to be equivalent to industry standards. The SVANOVA ELISA for *M. paratuberculosis* shows some promise of improvement on current North American testing methods. Additional evaluation of this test is necessary to discern its full capabilities.

References

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Comparison of Two Assay Techniques to Study *In Vitro* Phagocytosis of Bovine Granulocytes Using Flow Cytometry

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Introduction

The response of phagocytic cells to *Pasteurella haemolytica* is difficult to study *in situ*. Available assays to study the function of these cell types often do not accurately reflect the physiology of the cell in response to bovine pasteurellosis. This study compared granulocyte phagocytosis of fluorescent beads and fluorescent bacteria using flow cytometry. Development of an *in vitro* assay would provide a model to study nutrient and pharmaceutical effects on the immune response to *Pasteurella* infections. It would also allow for future studies of the pathogenesis of not only bovine pasteurellosis but other diseases.

Materials & Methods

Pasteurella haemolytica was cultured, harvested and conjugated with 5,6-carboxyfluroescein, succinimidyl ester (5,6-FAM-SE). Labeled bacteria was quantitated using a flow cytometric technique and snap frozen at –70°C until time of assay. Whole blood was collected via jugular venipuncture into 10 ml sodium heparin Vacutainer™ tubes on three consecutive days from 10 bull calves, 10 heifer calves, and 10 bull calves respectively. Blood obtained from all 30 calves was used to test two *in vitro* phagocytic assay techniques.

Isolated leukocyte cell populations were prepared and stimulated, in triplicate, with fluorescent-labeled synthetic beads and labeled *P. haemolytica* to measure phagocytic cell activity. Uptake of the fluorescent polystyrene beads and 5,6-FAM-SE-conjugated bacteria by phagocytic cells was evaluated by flourescent emission using a Coutler EPICS XL flow cytometer. Variance components were estimated separately for each assay. The MIXED procedure of the SAS system was used to fit a random effects model and the GLM procedure was used to perform an analysis of variance comparing percent labeled cells for each assay.

Results and Conclusions

The bacteria assay labeled a higher percentage of cells, at 44.9%, than the bead assay at 19.4% ($P<0.001$). Estimates of variance components, including day, calf and sampling, are shown in Table 1. The higher value measured for uptake of bacteria, and greater variability of the bacteria assay, caused a large and non-constant lack of agreement between the two assays.

Table 1. Variance components of percent labeled cells for bead and bacteria phagocytosis assays.

Source of variation	Variance[z]		P-value[y]	
	Bead	Bacteria	Bead	Bacteria
Day	1.5±5.3	13.2±25.1	0.3902	0.2999
Calf	23.2±10.4	96.4±32.2	0.0130	0.0014
Sampling	41.8±7.6	63.5±11.6	0.0001	0.0001

[z]Each value is the variance estimate ± standard error.
[y]P-value of the Z-test of the hypothesis that the variance=0 .

We conclude from this study that the labeled bacteria assay is more representative of the physiologic response of the animal, and therefore a more sensitive indicator of phagocytic cell activity in the animal. Applications of this technique versus the bead assay would include animal selection based on cell function as a predictor of performance or nutrient or drug studies to measure cell response during an inflammatory process.

Evaluation of Nitric Oxide Production by Bovine Alveolar Macrophages

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Introduction

Expression of inducible nitric oxide synthase (iNOS) and production of nitric oxide (NO·) is a key defensive response of rodent macrophages against taxonomically diverse infectious agents *in vitro* and *in vivo*.[1,4] Bovine alveolar macrophages (bAM) express iNOS in response to stimuli known to be present in pneumonic lung, but the role of NO· production in infectious pneumonia of cattle remains unknown.[2,3,4] This work was designed to: i) evaluate the microbicidal activity of NO· against *P. haemolytica* A1, ii) determine if virus infection of bAM alters subsequent NO· production, and iii) determine if activation of bAM for NO· production alters macrophage permissiveness for subsequent viral infection.

Materials and Methods

IFN-γ and endotoxin were used to activate bAM. NO· production and cell viability were measured by the Greiss reaction and MTT assays, respectively. Bacterial killing and viral titers were measured by limiting dilutional analysis. Killing of *P. haemolytica* was measured after exposure to reactive nitrogen oxides (RNO) generated by S-Nitroso-N-acetyl-D,L-penicillamine (SNAP) and 3-mopholinosydnonimine (SIN-1) or activated macrophages. NO· production and cell viability was measured in macrophages infected with cytopathic bovine virus diarrhea virus (BVD), bovine herpes virus type 1 (BHV) and parainfluenza type 3 (PI3). Macrophages activated for NO· production were subsequently infected with these viruses, and viral titers measured after an additional 48 hours in culture.

Results and Conclusions

Chemically-generated RNO kill *P. haemolytica* in a dose-dependent fashion. bAM kill leukotoxin-deficient *P. haemolytica*, but prior stimulation for NO· production abrogates this effect. BHV and BVD infection depresses NO· by affecting bAM viability. PI3 depresses NO· production, apparently by altering bAM function. Prior stimulation of bAM for NO· production did not affect the