

Report on the North American Symposium on Bovine Respiratory Disease

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The North American Symposium on Bovine Respiratory Disease was held at the Hilton Inn in Amarillo, Texas on September 7-9, 1983. The Symposium was sponsored by the Texas Agricultural Experiment Station and the NC-107 Technical Committee on bovine respiratory disease.

I will abstract for you some of the data presented at the symposium. One of the speakers was Dr. Bruce Rosenquist of the University of Missouri, and his topic was *Viruses as Etiologic Agents in Bovine Respiratory Disease*. Some of his comments were as follows.

Most acute respiratory disease in humans is caused by viruses, and, similarly in cattle, viruses are blamed for the majority of cases of acute bovine respiratory disease (BRD).

We are often too reluctant in veterinary medicine to accept some viruses as pathogens, sometimes insisting on fulfilling Koch's postulates as originally written. It has been stated that "insistence on their fulfillment before causality is accepted with a new agent in relation to a disease should be abandoned".

Under experimental conditions, it may be difficult or impossible to include all factors involved in production of clinical signs of BRD after exposure to a suspected pathogen—yet this should not exclude a virus from being considered a pathogen under the right conditions.

We need a nationwide (more accurately multiregional) effort to clarify the etiology of BRD. A true picture of the role all these agents play in the various clinical syndromes of bovine respiratory disease in the United States will eventually require nationwide, well-coordinated studies.

All recognize the "Big 3", IBR, BVD, and PI-3 as being ubiquitous bovine viruses, but there are other viruses we generally ignore.

Adenoviruses

SEROTYPES—Eight serotypes of bovine adenoviruses (BAV) are recognized (9th candidate) in the world. Not all have been isolated in the United States, nor are all associated with disease.

IMPORTANCE—It appears that type 3 is the most important BAV pathogen in the BRD complex in the United States.

INCIDENCE—Where studied, adenoviruses appear to be widespread in the cattle population. In some studies, the majority of adult cattle have been seropositive to types 1, 2, 3, or 4, and in some instances up to 100% were seropositive.

PATHOGENICITY—In the field, pneumonia or pneumo-

enteritis has been associated with BAV-3 infection in young calves and in feedlot cattle with acute BRD. The BAV-4 has been isolated from a week-old calf with pneumoenteritis, and an 8-month old bull with fever and respiratory disease. The BAV-7 has similarly been isolated from calves with pneumonia or pneumoenteritis.

SIGNS—These may include fever, lacrimal and nasal discharge, diarrhea, conjunctivitis, and coughing. Experimental infections with some serotypes produce similar signs.

Respiratory Syncytial Virus

SEROTYPES—One serotype is recognized.

IMPORTANCE—This virus is an important cause of respiratory disease in cattle. Infection with bovine respiratory syncytial virus can explain a number of previously undiagnosed outbreaks of BRD in the United States.

INCIDENCE—Serologic surveys indicate widespread infection amongst United States cattle. Where such testing has been done, the number of seropositive cattle has ranged from 38% to 100%. Approximately 88% of Missouri beef and dairy calves less than 1 year of age are seropositive.

PATHOGENICITY—Experimentally, mild signs of BRD can be produced—fever, rhinitis, with or without cough. Some reports indicate more severe experimental disease.

SIGNS—Reported signs include fever, rapid respiratory rate, cough, and nasal discharge.

Reoviruses

SEROTYPES—Three.

IMPORTANCE—Don't know.

INCIDENCE—Reovirus neutralizing.

PATHOGENICITY—Experimental infection of calves, especially with type 1, has produced mild respiratory illness and lung lesions.

Rhinoviruses

IMPORTANCE—In some cases they are the only viruses demonstrable in calves with BRD. Human rhinoviruses are the most common viruses isolated from people with common colds, and also occasionally cause more serious illnesses such as bronchitis.

INCIDENCE—These "nose viruses" are ubiquitous among the United States cattle population wherever incidence has

been studied. Seropositive rates to type 1 bovine rhinovirus (neutralizing antibody) recorded include 95% in Maryland cattle and 71% in Iowa calves. Approximately 95% of Missouri beef and dairy calves less than 1 year of age are seropositive; the figure approaches 100% by 12 months of age.

SIGNS—Fever, lachrimation, nasal discharge, cough and increased respiratory rate may occur.

Enteroviruses

SEROTYPES—Seven.

IMPORTANCE—Not been determined.

INCIDENCE—These viruses are widespread.

Another speaker was Dr. Glenn Frank of NADL, Ames, Iowa, whose topic was *Bacteria as Etiologic Agents in Bovine Respiratory Disease*. Some of his comments were as follows:

The nasal flora of cattle undergoing acute BRD will often contain *P. haemolytica* serotype 1 as the predominant species. *P. multocida* is widely distributed and is often found in the nasal passages of both healthy calves and those undergoing BRD. *H. somnus* is isolated from cattle with bronchopneumonia and fibrinous pneumonia, but its involvement in typical BRD is unclear.

At least 12 mycoplasma species have been isolated from the respiratory tracts of cattle. The role of mycoplasmas in BRD has not been determined.

From all observations and experimental evidence *P. haemolytica* and *P. multocida* are the most important bacteria involved in BRD.

In a 2-year study of the development of BRD in calves going through the marketing process, Dr. Frank made several key observations.

Few calves at the farm of origin carried detectable numbers of *P. haemolytica* in the nasal passages. On the farm of origin, almost all *P. haemolytica* isolates were serotype 2. The nasal carrier rate increased at the auction barn and was markedly high at the feedyard, after the calves had been transported 1,600 km. Shortly after arrival at the feedyard, during the episodes of acute BRD, 80-100% of the *P. haemolytica* isolates from the nasal passages were serotype 1. Only serotype 1 was isolated from the lungs of the calves that died from acute BRD.

The sudden rapid proliferation of *P. haemolytica* in the nasopharynx of stressed or sick calves is a phenomenon that was observed only recently. Before serotyping studies were done routinely, the concept was that the same *P. haemolytica* as that involved in BRD could be readily isolated from the nasal passages of healthy calves.

PASTEURELLA HAEMOLYTICA serotype 1 is isolated almost exclusively from the nasal passages of calves suffering from BRD at the feedyard and from pneumonic lungs. The rapid proliferation in the nasal passages can occur after the stress of transportation or as a result of virus-

induced clinical illness.

Many important research questions remain about pasteurilla.

1. Where and how does *Phaemolytica* serotype 1 live in the upper respiratory tract of the healthy calf?
2. What mechanism allows the rapid selected proliferation of *P. haemolytica* serotype 1 in the nasal passages?
3. What are the interactions of *P. haemolytica* serotype 1 with the host lung?
4. What are the best methods for determining the efficacy of immunizing products?
5. Finally, if *P. haemolytica* serotype 1 were completely eliminated, would BRD be the same problem as exists at present? Would the void left by *P. haemolytica* serotype 1 be filled by another serotype of *P. haemolytica* or by another bacterium?

Another speaker was Dr. James A. Roth, of Iowa State University, whose topic was *Immunosuppression and Immune Modulators*.

The purpose of Dr. Roth's report was to review the evidence that stressors, viruses, and bacteria associated with BRD impair host defense mechanisms, to characterize the nature of the impairments and to discuss the potential for immunomodulators to reduce the losses associated with BRD.

Immunosuppression by Stress

Several stressors (castration, dehorning, weaning, handling, etc.) associated with the movement of cattle into feedlots have been proven to result in increased plasma cortisol concentration. There is evidence that high plasma cortisol concentrations affect several aspects of the host defenses. Increased plasma cortisol also impairs certain aspects of neutrophil function. Perhaps the most important effect is to inhibit the emigration of neutrophils into the tissues at sites of inflammation.

In addition to inhibiting neutrophil accumulation in the tissues, cortisol also inhibits the activity of the hydrogen peroxide-halide-myeloperoxidase anti-bacterial system (probably by inhibiting degranulation) and increases the rate of random migration of neutrophils. These effects may be due to an inhibition of microtubule function in the neutrophil. Increased plasma cortisol due to ACTH administration in cattle does not measurably impair the ability of neutrophils to ingest *staphylococcus aureus*, generate the burst of oxidative metabolism or mediate antibody-dependent cell-mediated cytotoxicity. Dexamethasone, a more potent glucocorticoid does inhibit these activities of bovine neutrophils at pharmacologic dosages.

The effects of glucocorticoids on macrophage function in the bovine has apparently not been studied. In other species the macrophage is generally sensitive to glucocorticoids and has reduced phagocytic and bactericidal capabilities.

The suppression of host defense mechanisms (probably phagocytic cell activity) by glucocorticoids early in virus

infection apparently facilitates viral replication. Recrudescence of the IBR virus with dexamethasone is a well known phenomenon.

Immunosuppression by Viruses and Mycoplasma

The viruses associated with the bovine respiratory disease complex usually produce only mild lesions and clinical signs when inoculated into normal cattle. Their real importance in BRD is probably due to their detrimental effect on pulmonary defense mechanisms which facilitates secondary infection by bacteria.

There are several types of evidence commonly cited to suggest that respiratory viruses are immunosuppressive.

1. Epidemiologic investigation may reveal that infection with, or serologic conversion to, a particular virus is associated with a higher incidence of bacterial pneumonia.
2. Inoculation of experimental animals with a virus may facilitate lung colonization by a subsequent bacterial challenge.
3. Viral infection in the animal may result in defects in host defense mechanisms evaluated by *in vitro* procedures.
4. Incubation of a virus with lymphocytes or phagocytes *in vitro* may alter cellular functions.

Impairment of Host Defense Mechanisms by Bacteria

P. HAEMOLYTICA elaborates a cytotoxin which is toxic for bovine neutrophils, lymphocytes, and macrophages.

The capsule of Type A *P. multocida* has been demonstrated to inhibit the ingestion of the organism by bovine neutrophils. The capsular material was found to inhibit the ability of neutrophils to ingest *staphylococcus aureus* and to iodinate protein through the myeloperoxidase-hydrogen peroxide-halide antibacterial system.

H. somnus was also found to have heat extractable surface material that was capable of inhibiting *S. aureus* ingestion and iodination by bovine neutrophils without affecting neutrophil oxidative metabolism.

Immunomodulation in Bovine Respiratory Disease

Levamisole is a widely used anthelmintic for cattle which has received a lot of attention as a potential immunomodulator. Levamisole has been shown to have its greatest effect on cells of the immune system which aren't functioning properly, with little or no effect on normal cells. The effects of levamisole on the immune system are apparently heavily dependent upon the dosage used, time of administration and condition of the animal. Most of the research on levamisole as an immunomodulator has been conducted in species other than cattle. Dr. Roth reviewed research conducted on cattle relative to BRD.

It has been reported that levamisole (8 mg/kg, sc) given simultaneously with IBR vaccination (IM) had a moderate

inhibitory effect on the subsequent antibody response.

In one report it was found that levamisole (6 mg/kg, sc) given at the same time as intranasal vaccination with attenuated IBR virus resulted in a moderate decrease in the antibody response to IBR virus, a decrease in the lymphocyte blastogenic response to IBR antigen, and a decrease in direct lymphocyte cytotoxicity for IBR infected target cells. In a later report, it was found that an identical dosage of levamisole given at the time of vaccination or 7 days later produced an enhanced antibody response to IBR virus.

Others reported that levamisole administration (10 mg/kg, orally) did not affect neutrophil numbers in normal or shipped-stressed cattle. Still others evaluated the effects of levamisole on selected parameters in cattle experimentally infected with BVD virus. The cattle were subjected to transportation stress and then inoculated with a cytopathic strain of BVD virus 24 hours later. The calves displayed mild clinical signs on day 7 post-inoculation. Beginning on day 7 one-half of the animals were given levamisole (2.0 mg/kg, sc) daily for 3 days. No differences in severity of infection, virus shedding, speed of recovery or antibody response was detected between levamisole treated and untreated animals. The total number of lymphocytes in the peripheral blood of levamisole treated cattle was higher than in the non-levamisole-treated cattle, but this difference also existed before infection.

In an attempt to determine if levamisole was capable of preventing or reversing some of the effects of glucocorticoids on the immune system, levamisole was administered at six different dosage levels to dexamethasone treated cattle (Roth and Kaeberle, submitted for publication). Dexamethasone (0.4 mg/kg, IM) and levamisole (0.5, 1.0, 2.0, 4.0 or 8.0 mg/kg orally) were administered daily for 3 days. Another group of animals received the 3 day treatment regimen with dexamethasone and a single 6.0 mg/kg dose of levamisole (the recommended anthelmintic dose) on the first day of dexamethasone administration. Levamisole had no apparent consistent ability to normalize lymphocyte blastogenic responsiveness (to the mitogens PHA, Con A, or PWM; or in a oneway mixed lymphocyte reaction) or to normalize neutrophil function (random migration, nitroblue tetrazolium reduction, iodination or antibody-dependent cell-mediated cytotoxicity) in dexamethasone-treated cattle.

Perhaps the most convincing type of experiments for determining if levamisole may be effective in reducing the losses due to shipping fever are those experiments in which large numbers of cattle arriving at a feedlot are treated with levamisole and the morbidity and mortality in these animals are compared to that in closely matched, co-mingled control animals. In one such experiment involving 1,464 feedlot cattle, a single treatment with levamisole phosphate (8 mg/kg, sc) was associated with a reduced incidence of shipping fever as compared to the morbidity observed in cattle treated with either thiabendazole (66 mg/kg, orally) or levamisole hydrochloride (8 mg/kg, orally). This beneficial

effect was only observed in those animals that became ill between approximately day 8 through 30 after levamisole administration.

The efficacy of levamisole phosphate in reducing morbidity due to shipping fever was also evaluated in another experiment involving a total of 2,241 cattle in six trials located in different states. In each trial 300 to 470 animals were processed and randomly allotted to control or treated groups. The treated group received a single 6 mg/kg injection (sc) of levamisole phosphate. There was no statistically significant difference in incidence of respiratory disease or in mortality between the two groups. The levamisole treated group did have an improvement in average daily gain and in feed efficiency; probably due to the removal of worms.

In summary, levamisole has, on occasion, produced results which could be interpreted to be beneficial to animals affected with or susceptible to shipping fever. More often, negative results have been found. The cumulative results to date are insufficient to recommend the use of levamisole as an effective immunomodulator in bovine respiratory disease.

Thiabendazole, like levamisole, is a commonly used anthelmintic in cattle which has been reported to have immunomodulating properties. At relatively low dosages (2 mg/kg and 20 mg/kg) thiabendazole has been observed to enhance immune responsiveness in mice while at high dosages (200 mg/kg, 1,000 mg/kg, and 2,000 mg/kg) it has been reported to be immunosuppressive. Also like levamisole, the immunomodulating activity of thiabendazole was observed to be the most pronounced in immunosuppressed animals.

Thiabendazole was evaluated for its ability to prevent or reverse some of the effects of glucocorticoids on the bovine immune system using a protocol similar to that described for levamisole. Dexamethasone (0.04 mg/kg, IM) and thiabendazole (0, 1, 3, 6, 12, 25, 50, or 100 mg/kg, orally) were administered concurrently each day for 3 consecutive days. Thiabendazole administration in the dosage range from 1.0 to 25 mg/kg was associated with a significant enhancement of lymphocyte blastogenic responsiveness to PHA, Con A PWM, and allogeneic cells in dexamethasone-treated cattle. This immunonormalizing effect was not observed at the 50 or 100 mg/kg dosage levels or when the thiabendazole treatment was initiated 24 hours prior to dexamethasone administration. Thiabendazole did not produce a consistent significant normalization of any parameter of neutrophil function in dexamethasone treated cattle (random migration, nitroblue tetrazolium reduction, iodination, or antibody-dependent cell-mediated cytotoxicity).

Since thiabendazole had some activity in normalizing lymphocyte blastogenesis in dexamethasone-treated animals an experiment was undertaken to determine its effect on antibody responses in stressed cattle. Fifty-one calves were divided into a control group and a thiabendazole treated group. Animals in both groups were stressed by

weaning, castration, placement in a feedlot, and injection of antigens (equine ferritin, tetanus toxoid, and killed brucella abortus strain 19) on the day that thiabendazole therapy was started. Thiabendazole administered orally for 5 consecutive days at a dosage of 20 mg/kg did not enhance the antibody response to any of the antigens. Thiabendazole treatment did significantly inhibit the antibody response to

B. abortus.

The sum of the evidence concerning levamisole and thiabendazole is that the dosage, frequency, and timing of administration relative to the immunosuppressive event are critical to obtaining immunorestoration. These factors will probably prevent either of these compounds from ever being an effective immunomodulator on a practical basis in bovine respiratory disease. They do serve as useful prototypes in the search for practical immunomodulating compounds.

After reading Dr. Roth's report I concluded that wormers ought to be purchased for their value as wormers.

Another speaker was Dr. Lorne Babiuk of the University of Saskatchewan, Saskatoon, Saskatchewan, Canada. His topic was *Experimental Models in Cattle*.

Models of Bacterial Pneumonia

I. DIRECT DEPOSITION OF PASTEURELLA HAEMOLYTICA INTO THE LUNG

It is possible to induce pneumonia in cattle by depositing bacteria directly into the lung by intratracheal or transthoracic injection. These methods, and modifications of them, bypass the defense and clearance mechanisms of the upper respiratory tract (URT) and therefore they overcome the requirement for proliferation and colonization of the URT. Furthermore, they are most consistent when large quantities of log-phase bacteria are inoculated. Since log-phase cultures produce large quantities of cytotoxin, this could mean that a certain amount of cytotoxin is necessary to impair host defense mechanisms sufficiently to allow the bacteria to become established.

II. MODELS WHICH IMPAIR HOST DEFENSES

It is possible to favor bacterial growth by interfering with the host defense mechanisms by a variety of methods. For example, this can be achieved by creating lung edema and by chemical or viral immunosuppression.

Factors Influencing Colonization of the Lung

- 1) VIRUS INFECTIONS - ALTER
 - mucociliary clearance
 - bronchotracheal secretions
 - bacterial adherence - fibronectin
 - local iron levels
 - macrophage functions
 - neutrophil functions
 - lymphocyte functions

2) *RESPIRATORY TRACT ENVIRONMENT*

- levels of FE++ and ZN++
- mucosal surfaces - mucus
 - quantity
 - quality
- lysozyme
- antibody

3) *BACTERIAL CHARACTERISTICS*

- cytotoxins (s)
- capsules
- fimbriae
- enzymes
- cation sequestering mechanisms
- endotoxin
- plasmids

Another speaker was Dr. Donald Morgan from Plum Island, New York, and his topic was *Biological Synthesis of Subunit Vaccines*. I've just lifted several statements from his summary to share.

Recent advances in biotechnology hold great promise for veterinary medicine, particularly in the area of vaccine development.

Peptide vaccines eliminate the danger of the spread of contagion and assure the quality and quantity of vaccine needed for disease control programs.

Recombinant DNA technology and organic synthesis provide alternate means of production with the former having an advantage with large polypeptide immunogens.

Dean Robert Kahrs of the University of Missouri made *Projections into the Future* and summarized some remarks at the conference.

It was reported that only 39% of available management knowledge is currently utilized by the beef industry and that 83% of cattlemen raise less than 100 calves.

Biological products need better care and handling and there is much room for improvement in their administration. The United States' biologic industry is actually two industries, a regulated industry, dedicated to product quality, long term research and product development and an unregulated industry, operating under a variety of standards.

We also learned that prevention of death and disease in feeder cattle is very desirable, but in the final analysis,

success of respiratory disease control programs will be measured by improved feed efficiency and demonstrable improvement in average daily weight gain.

FUTURE RESEARCH—Here are areas of study which may be profitable in the future. Immunopathological studies on cattle are needed to determine if pulmonic *pasteurellosis* is an immunologically mediated lesion. Studies are needed on the alleged immunosuppression of BVD virus; on bovine respiratory syncytial virus, *hemophilus somnus*, and on the neglected adnoviruses, rhinoviruses, enteroviruses and non-IBR herpes viruses.

The subject of immunomodulation in cattle is a fertile field for future research. It will be necessary to study the pathophysiology of stress and detail its effects on the bovine immune system and concurrently to study and identify the action of stress modifying drugs with the ultimate idea of a rapid delivery system for products to reduce the contribution of stress to bovine respiratory disease.

Subunit vaccines, be they chemically synthesized, genetically engineered or developed through detergent cleavage of infectious agents, must be developed to meet the burgeoning needs for safe, pure, potent and effective biologic products.

Funding

The big question facing the future of bovine respiratory research is funding. The USDA must quadruple its budget for this area.

Cattle producers must pioneer in an area they have neglected, that is, financing the research for which they clamour so loudly. They need to develop a check-off for research and need to increase their lobbying for this area. They must understand there is no free lunch and no quick fix. If they want quick answers for multicomplex problems, they must be prepared to support required research.

The time is right for the development of a national institute of veterinary research. The day when the USDA can handle the research necessary to overcome a problem like respiratory disease is gone because the USDA is too plant oriented, too heavily involved in regulatory quagmires and too closely related to and controlled by agricultural policies.